From DEPARTMENT OF MEDICAL BIOCHEMISTRY AND BIOPHYSICS
Karolinska Institutet, Stockholm, Sweden

INVESTIGATION OF T CELL DRIVEN ARTHRITIS INDUCED BY MANNAN IN ZAP70 MUTATED SKG MICE

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Stockholm 2016

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB

ISBN 978-91-7676-374-2

Investigation of T cell driven arthritis induced by mannan in ZAP70 mutated SKG mice

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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À Jean et Gemma qui malheureusement nous ont tous deux quitté au courant de cette aventure qu'était le doctorat à l'étranger. Je crois qu'ils auraient été fiers du résultat.

ABSTRACT

Autoimmune diseases are particularly challenging to study in human due to their complex multifactorial nature. In rheumatoid arthritis (RA), self-reactivity directed against joints leads to pain, tissue destruction and eventually invalidity if left untreated. Although there have been great advances in therapies over the past decades, there is still no cure. In this context, animal models are essential to improve our understanding of RA, identify new therapeutic targets and also evaluate new drugs. Unfortunately, no single model can perfectly mimic the human pathology. Hence, using a combination of independent models is critical to validate findings.

The SKG arthritis model is a novel murine model in which defective central tolerance, due to reduced TCR signaling from a mutation in *ZAP70*, leads to chronic T cell driven arthritis. The understanding of the SKG model has grown over the years, and its use in drug discovery is now also steadily increasing. In this thesis, the SKG model is carefully characterized regarding innate immunity activation by mannan (paper I), the influence of genetic backgrounds (paper II), and the role of collagen type II (CII) as a potential self-antigen (paper III). Finally, the SKG model is used to study the regulation of arthritis by reactive oxygen species (ROS) (paper IV).

Stimulation of innate immunity is essential to activate T cells and trigger chronic arthritis in SKG mice. In specific pathogen free (SPF) animal facilities, this can be achieved by a single injection of mannan, a polysaccharide extracted from *S. cerevisiae*. **Paper I** focuses on innate activation and the acute skin and joints inflammation it triggers in non-SKG mice. This manuscript highlights the importance of IL-17, potentially originating from γδ T cells, giving significant insight in the early phase of SKG arthritis preceding αβT cells involvement.

Prior to this thesis, the SKG model had only been described on the BALB/c genetic background. In **paper II**, the mutation was backcrossed over more than 10 generations on B10.Q and B6.Q genetic backgrounds. Arthritis susceptibility is unaffected by the genetic backgrounds investigated, allowing the use of the SKG model on these common strains. This finding opens many possibilities in terms of crosses with genetically modified strains.

Using a series of CII-specific TCR transgenic strains, **paper III** investigates the role of CII self-reactivity in the SKG model. Although CII reactivity is spontaneously observed in arthritic SKG mice, further increasing CII self-reactivity does not affect arthritis. In fact, restricting T cells repertoire to CII reactivity abolishes susceptibility to arthritis. CII reactivity is therefore not essential in SKG arthritis, and is likely a consequence of joints destruction.

Finally, **paper IV** uses the SKG model to dissect the mechanisms of immunoregulation by ROS in autoimmunity. Thymic selection and T cells activation are unaffected by ROS deficiency. Although concentrations of anti-CII antibodies are higher in ROS deficient mice, the sera itself is not pathogenic. Instead, it is ROS production in the periphery, in particular from macrophages, which mediates immunosuppression in SKG arthritis.

LIST OF SCIENTIFIC PAPERS

I. **Mannan induces ROS-regulated, IL-17A–dependent psoriasis arthritis-like disease in mice.**
Ia Khmaladze, Tiina Kelkka, Simon Guérard, Kajsa Wing, Angela Pizzolla, Amit Saxena, Katarina Lundqvist, Meirav Holmdahl, Kutty Selva Nandakumar, Rikard Holmdahl.
Proc Natl Acad Sci USA. 2014 Sep 2;111(35):E3669-78.

II. **The SKG mutation in ZAP-70 also confers arthritis susceptibility in C57 Black mouse strains.**
Simon Guérard, Margherita Boieri, Malin Hultqvist, Rikard Holmdahl, Kajsa Wing.
Scand J Immunol. 2016 Jul;84(1):3-11.

III. **T cell specificity against galactosylated CII enhances collagen induced arthritis in SKG mice but does not affect adjuvant induced arthritis.**
Simon Guérard, Rikard Holmdahl, Kajsa Wing.
Manuscript.

IV. **ROS regulate innate but not adaptive inflammation in ZAP70 mutated SKG arthritic mice.**
Simon Guérard, Rikard Holmdahl, Kajsa Wing.
Am J Pathol. 2016 Jul 14. pii: S0002-9440(16)30209-7. doi:10.1016

CONTENTS

LIST OF ABBREVIATIONS

ACPAs	Antibodies to citrullinated protein antigens
APC	Antigen-presenting cell
CGD	Chronic granulomatous disease
CIA	Collagen induced arthritis
CII	Collagen type II
CPP	Cyclic-citrullinated peptides
DAMP	Damage-associated molecular pattern
DC	Dendritic cells
DN	Double negative
dLN	Draining lymph node
DMARD	Disease-modifying antirheumatic drugs
DP	Double positive
GPI	Glucose-6-phosphate isomerase
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
Ncf1	Neutrophil cytosolic factor
NK	Natural killer
NOX2	NADPH oxidase 2
PAD2	Peptidylarginine deiminase 2
PAMP	Pathogen-associated molecular pattern
PIA	Pristane induced arthritis
PRR	Pattern recognition receptor
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
RA	Rheumatoid arthritis
RAG	Recombination-activating genes
RF	Rheumatoid factor
ROS	Reactive oxygen species
SP	Single positive
SPF	Specific pathogen free facilities
Syk	Spleen tyrosine kinase

TCR	T cell receptor
Th	T helper cells
TLR	Toll-like receptor
Treg	Regulatory T cell
VEGF	Vascular endothelial growth factor
ZAP70	Zeta-chain associated protein kinase 70kDa

1 IMMUNOLOGY

1.1 WHAT IS IMMUNOLOGY?

Immunology is the medical science studying the immune system in both health and diseases [1, 2]. Although most of its history is fairly recent, discoveries in immunology have already had tremendous impact in medicine. Originally, studies in immunology focused on infectious diseases, leading to major breakthroughs, such as vaccination, which completely changed the history of medicine. In more recent years, findings in immunology have greatly improved treatments for various inflammatory diseases (e.g. asthma, rheumatoid arthritis) [3]. The importance of immunology is now spreading to other fields, such as oncology, wound healing and even cardiology [4-8].

The immune system is composed of various organs and cells patrolling our body, and is responsible to protect the organism against diseases. The two main functions of the immune system is first to detect and recognize threats to the organism, and then to neutralize and eliminate such threats. These threats can either be of foreign origins (e.g. bacteria, viruses, fungus and parasites) or of intrinsic origin (e.g. tumors). The immune system is thus essential to protect us against both infections and cancers. Hereditary or acquired shortcoming in its functions lead to immunodeficiency, a state of high susceptibility to various infections and malignancies [9-11]. On the other hand, hyperactivity of the immune system can cause many diseases, including autoimmune diseases.

For these reasons, a tight regulation of the immune system's activity is crucial, and a good understanding of these mechanisms by the biomedical community has far reaching implications. A practical and frequent approach to describe the immune system is to divide it in two systems: innate immunity (section 1.2) and adaptive immunity (section 1.3) [2]. These two systems are however not independent. In fact, they support and influence each other.

1.2 INNATE IMMUNITY

Innate immunity encompasses mechanisms to both limit entry of pathogens in the organism and to eliminate such pathogens. The innate immune system is characterized by its ability to react rapidly to threats. This is possible because it relies on recognition of infectious agents through pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) rather than specific antigen recognition requiring antigen processing and subsequent clonal expansion like in T cells and B cells responses (section 1.3).

1.2.1 Epithelial surfaces

Serving as frontiers between the organism and a hostile environment, the skin, the gut and the lungs are all frequently exposed to infectious agents. Epithelial surfaces of these organs serve as the first line of defense since pathogens need to bypass them to reach internal organs. Through mechanical (e.g. tight junctions) and chemical mechanisms (e.g. fatty acids,

digestive enzymes, antibacterial peptides), these organs limit access, implantation and short term survival of infectious agents [12, 13]. Moreover, the presence of commensal microflora in the gut and the skin limits implantation of pathogens by direct competition for space and nutrients [14, 15]. The impact of the gut microflora on the immune system is however much more complex than this, as it was shown to affect the development of the immune system, and even to modulate the immune response [16-18].

1.2.2 Immunosurveillance

Despite these mechanisms, some pathogens are bound to breach these protective layers by different routes, for instance following skin injury. Immunosurveillance is the constant surveillance by the immune system for threats to the organism such as infectious agents and malignant cells. This is primarily done by phagocytes, such as monocytes, macrophages and dendritic cells (DC), which discriminate self from non-self through PAMPs recognition, and danger through DAMPs recognition [19]. Natural killer (NK) cells also participate in immunosurveillance, however their activation rely mostly on the missing-self mechanism, and the presence of DAMPs [20, 21].

1.2.2.1 Pathogen-associated molecular patterns (PAMPs)

PAMPs are highly conserved molecular patterns specific to a family of pathogens, and which are usually essential for their survival. A classic example is lipopolysaccharides (LPS), an endotoxin found in the outer membrane of gram-negative bacteria [22].

1.2.2.2 Damage-associated molecular patterns (DAMPs)

In contrast, the danger model proposes that rather than discrimination of self and non-self, the immune system discriminates between safe and dangerous situations using "alarm signals" from injured or stressed tissues [23]. These include DAMPs, host molecules such as nuclear or cytoplasmic proteins, often released in the extracellular fluids upon cell necrosis [24]. A well-characterized DAMP is the chromatin-associated protein high-mobility group box 1 (HMGB1) [25, 26].

1.2.2.3 Mannan

An important PAMP for this thesis is mannan, a fungal polysaccharide extracted from the cell wall of yeast (e.g. *C. albicans* and *S. cerevisiae*). The structure consists of a backbone of α-(1,6)-linked mannose with α-(1,2) and α-(1,3) side chains [27]. Several receptors were found to bind mannan, including the mannose receptor, dectin 2, TLR4 and DC-SIGN [28-31]. It activates monocytes, macrophages and DCs to produce cytokines supporting mostly Th17 differentiation, and also to a lower extent Th1 [32-34].

1.2.2.4 Pattern recognition receptors (PRRs)

Both PAMPs and DAMPs are recognized by pattern recognition receptors (PRRs) [19, 24]. These receptors include toll-like receptors (TLRs), NOD-like receptors and C-type lectin receptors [35, 36]. Activation of PRRs is a key event leading to cytokines production

necessary to activate and modulate both innate and adaptive immunity to respond appropriately to various sources of threats.

Monocytes are among the innate cells expressing PRRs. Upon recognition of pathogens through the activation of PRRs, monocytes engulf them. Some monocytes then differentiate into macrophages [37]. Others will differentiate in DC and start migration towards the draining lymph node (dLN) [38]. During this transit, DC will further mature by upregulating co-stimulatory molecules such as CD80 and CD86 [39]. Upon arrival in the dLN, cell maturation is over and DC are able to present to T cells various antigens from the digested pathogen, while also expressing the necessary co-stimulatory molecules required to activate naïve T cells.

1.2.3 Antigen presentation

Antigen presentation by innate cells is an important link between innate and adaptive immunity. Every nucleated cell constantly presents intracellular antigens on major histocompatibility complex (MHC) class I [1]. This is a general mechanism of surveillance against intracellular infections and mutations. Down-regulation of MHC-I, for instance by virus as an escape mechanism, will lead to activation of NK cells due to the lack of inhibitory signal from self-MHC (the missing-self theory). Antigen-presenting cells (APC) also have the ability to present antigens from extracellular sources, for example phagocytosed bacteria, on MHC-II. This mechanism is important for the defense against extracellular infectious agents. Professional APCs, such as DCs, also express co-stimulatory molecules.

1.2.4 Macrophages

Macrophages are tissue resident cells with a wide variety of functions [2, 40]. In recent years, researchers have highlighted the complexity and diversity of the different macrophage populations. A common classification is the distinction between M1 and M2 macrophages [41]. Whereas M1 macrophages are implicated in host defense and are thus considered pro-inflammatory, M2 macrophages favor tissue repair and have potent anti-inflammatory proprieties [42]. Originally, the classification in classically activated M1 and alternatively activated M2 was proposed due to the parallel with the Th1/Th2 paradigm (section 1.3.7.2 - T helper lymphocytes), where M1 are associated to IFN-γ, and M2 to IL-4, IL-13 and IL-10 (Figure 1) [43, 44]. This classification is however not a true dichotomy, and polarization is partly reversible [43, 45]. More recent classifications generally also include wound-healing macrophages.

As mentioned previously, an important function of macrophages is immunosurveillance of tissues for threats, and the subsequent phagocytosis of pathogens. The cytokines and chemokines they release following the activation of PRR are essential to attract leukocytes from the blood, such as T cells, to help fight the infection. Due to their expression of MHC-II, macrophages can present antigens to activated T helper (Th) cells in inflamed tissue (section 1.3.7.2 - T helper lymphocytes). This interaction is essential to cytokines release from Th, which triggers the digestion of phagosomes by macrophages and causes an influx of effector

cells including neutrophils. Macrophages express the NADPH oxidase 2 (NOX2) complex, producing large amount of reactive oxygen species (ROS) which are used in the digestion of phagocytosed pathogens [46].

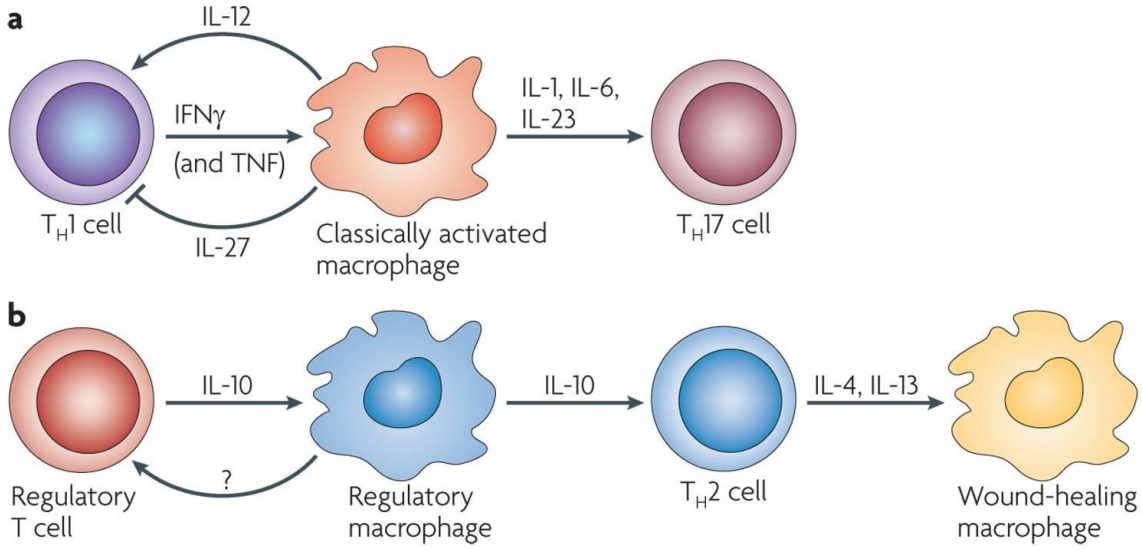

Figure 1 - Interactions between macrophages and T cells

Key mediators involved in macrophages-T cells interactions from classically activated macrophages (a), regulatory macrophages and wound-healing macrophages (b). Whereas pro-inflammatory Th1 and Th17 are associated to classically activated macrophages (M1), both Treg and Th2 are associated to regulatory and wound-healing macrophages (M2).

1.2.5 Neutrophils

Neutrophils are fast acting effector cells of the innate immune system. In healthy state, a large population of neutrophils already circulates in the blood to facilitate rapid deployment in inflamed tissues. Inflammation further increases the concentration of circulating neutrophils. Upon detection of inflammation (e.g. chemokines and cytokines), neutrophils rapidly migrate to inflamed tissue via adhesion molecules expressed on endothelial cells [47]. Chemokines gradients direct the cells towards the threats where neutrophils will attack pathogens [48]. The action of neutrophils is meant to be fast; therefore they store large amounts of mediators in granules.

Depending on the duration and strength of stimulation, they will release primary granules (azurophilic) containing myeloperoxidases, defensins, elastases and phospholipases (A2, C and D) [49, 50]. Phospholipases in primary granules are implicated in the synthesis of lipid mediators which are potent but short lived inflammatory mediators (e.g. LTB4 and PAF).

Secondary granules contain lactoferrin and lysozyme, whereas tertiary ones contain collagenases. Finally, neutrophils also produce large amount of ROS via the NOX2 complex [46, 51].

Enzymes and mediators released by neutrophils are very potent to fight infections, but they can also damage host tissues [52]. To prevent prolonged uncontrolled inflammation, the survival of neutrophils in inflamed tissues is limited as they undergo programmed cell death relatively quickly after leaving the blood [53]. Hence, a constant influx of neutrophils dependent on cytokines and chemokines from various sources (e.g. T cells) is required until the threat is fully neutralized.

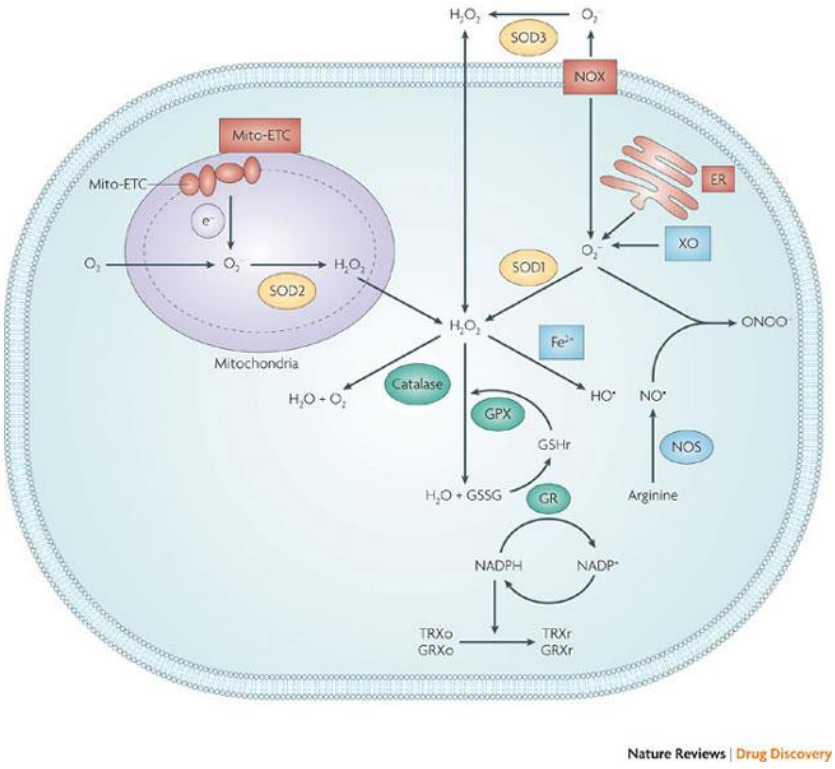

Figure 2 - Cellular redox biology

Cellular ROS originates from various sources (indicated in red). Superoxide (O_2^-) is generally the original free radical produced. It can then be converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD). H_2O_2 is relatively stable, membrane permeable and it can be converted to water by peroxiredoxins and glutathione peroxidase when in low concentrations, or to water and oxygen by catalase at high concentrations. In the presence of free heavy metal (such as Fe^{2+}), H_2O_2 can also be converted to hydroxyl radicals (HO•) through the Fenton reaction. HO• is highly reactive and toxic, reacting with lipids, proteins and DNA. Major ROS-scavenging enzymes are shown in green. GPX, glutathione peroxidase; GR, glutathione reductase; GRXo, glutaredoxin (oxidized); GRXr, glutaredoxin (reduced); GSHr, glutathione (reduced); GSSG, glutathione (oxidized); TRXo, thioredoxin (oxidized); TRXr, thioredoxin (reduced); XO, xanthine oxidase.

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1.2.6 Reactive oxygen species (ROS)

ROS are a group of highly reactive molecules derived from oxygen. They can originate from both exogenous and endogenous sources. Redox biology studies the physiological and pathological effects of the reduction–oxidation balance which depend on the location, duration and exact molecular nature of various ROS [54]. Oxidation of cysteine residues due to variations in the redox balance modifies proteins conformation and activity [55]. Cell permeability, reactivity, half-life and toxicity vary greatly among various ROS. Figure 2 presents the homeostasis of cellular ROS.

1.2.7 NADPH oxidase 2 (NOX2)

NOX2, also referred to as the phagocyte NADPH oxidase, is responsible for respiratory burst of neutrophils and macrophages [46, 56]. Cellular activation of neutrophils upon detection of microorganisms or pro-inflammatory mediators results in the activation of the NOX2 complex and the production of large amount of $O_2^{\cdot-}$. According to the current model of NOX2 activation, Ncf1 (also known as $p47^{phox}$) serves as organizer subunit. Upon phosphorylation, $p47^{phox}$ interacts with $p22^{phox}$, leading to the translocation of other cytosolic factors such as the activator subunit $p67^{phox}$ to the catalytic subunit $GP91^{phox}$ (Figure 3) [57]. Without the organizer subunit $p47^{phox}$, the NOX2 complex cannot recruit the activator subunit and is therefore dysfunctional [58]. Other NOX complexes have been identified in several other cell types, but NOX2 is by far the most potent.

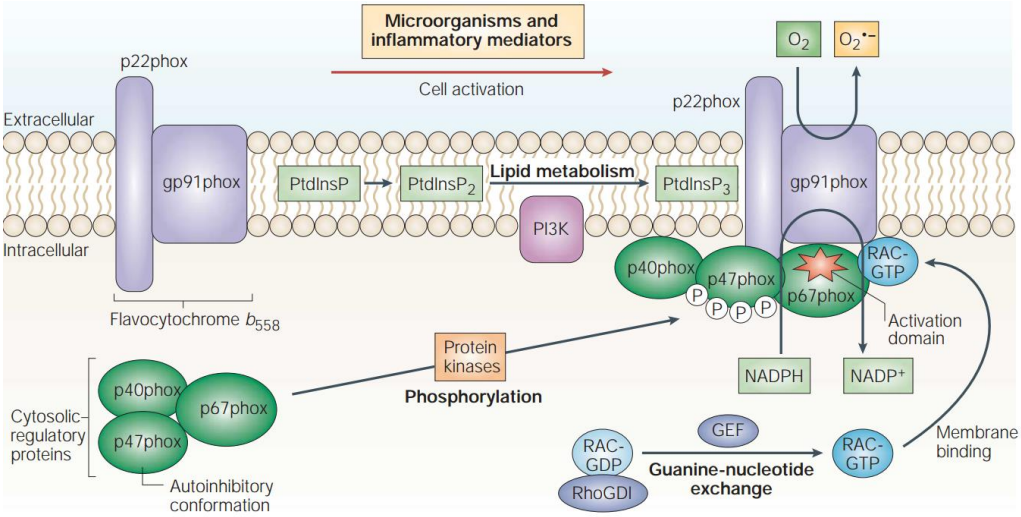

Figure 3 - Activation of the NOX2 complex

In resting neutrophils, the NOX2 complex is inactive. Upon cellular activation, phosphorylation of $p47^{phox}$ leads to the translocation of $p22^{phox}$ and $p67^{phox}$ to the catalytic subunit $gp91^{phox}$. The assembled NOX2 complex then generates high amounts of $O_2^{\cdot-}$.

1.3 ADAPTIVE IMMUNITY

Whereas innate immunity relies on recognition of group of pathogens through class-specific molecular motives, adaptive immunity is antigen-specific, meaning that it is slower due to the requirement for antigen processing and clonal expansion. The other key feature of adaptive immunity is memory [59]. The main components of adaptive immunity are B cells that produce antibodies, and T cells that either directly kill cells, produce cytokines to orchestrate the immune response or assist B cells in antibody production. The work in this thesis primarily concerns T cells; therefore they will be the focus of this section.

1.3.1 T cell development

In the bone marrow, the hematopoietic stem cells divide to give rise to the common lymphoid progenitor, which is the source of T cells, B cells, innate lymphoid cells and NK cells [2]. At some point during differentiation, some cells leave the bone marrow through systemic circulation and reach the thymus, a central lymphoid organ where T cells development and selection occur. Following signaling by stromal cells, these cells commit to T cell lineage [60]. After differentiation in the thymus, they start proliferating to generate the very high number of thymocytes required to have sufficient T cells output considering that approximately 97% of thymocytes undergo apoptosis during the followings steps of development [61, 62].

1.3.2 T cell receptor (TCR)

A key feature of adaptive immunity and T cells is the generation of a large repertoire of cells with unique specificity. This is possible because each T cell is restricted to a unique T cell receptor (TCR), with affinity for a specific antigen presented by a specific MHC molecule. The TCR is a membrane-bound heterodimer composed of either α- and β-chains (~95%), or γ- and δ-chains (~5%). Although they are both technically "T cells", the biology, the development and the functions of so called αβT and γδT cells are very different and should not be confused. Unless specifically mentioned, the term "T cells" is usually used in literature and in this thesis to refer to αβT cells, whereas γδT cells are always referred specifically as γδT cells.

1.3.3 V(D)J recombination

The large repertoire of TCR poses an interesting challenge: the number of potential unique TCR molecules is too large for them to be included as single genes in the genome. Instead, to create this vast library of unique TCR, a limited set of interchangeable genes are encoded in the genome. In thymocytes, the different regions of these genes are recombined in a process called V(D)J recombination during which the TCR loci are re-arranged in a semi-randomized process giving rise to different clones, each expressing a TCR with unique specificity [1, 63]. Figure 4 is a schematic representation of both β- and α-chain re-arrangements [64].

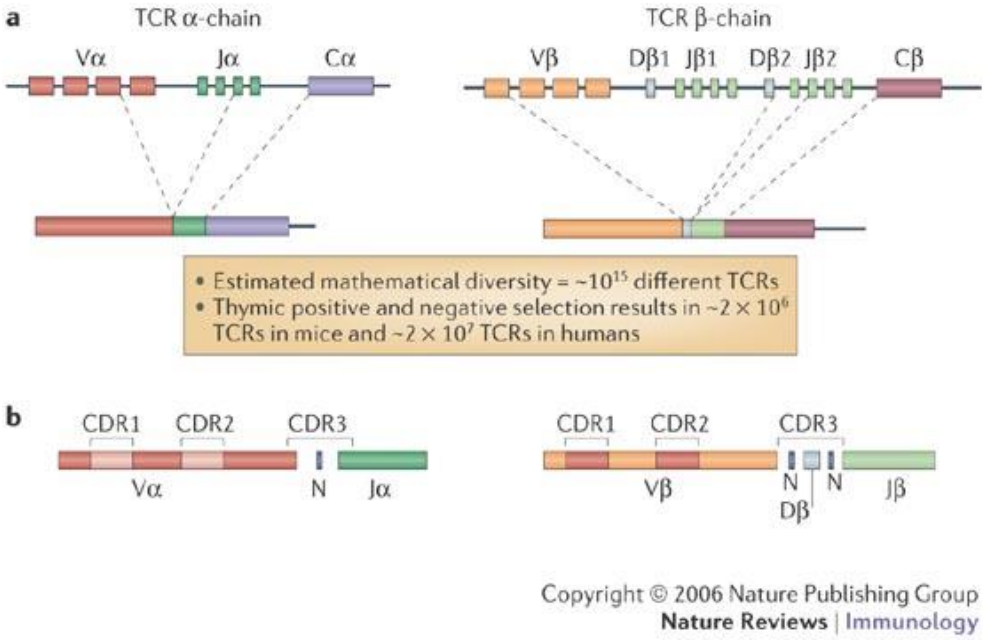

Figure 4 - V(D)J recombination of α- and β-TCR chains

Somatic gene recombination of variable (V), diversity (D) and junctional (J) gene segments of first the β-chain, and then the α-chain, generates the vast diversity of unique TCR. Complementarity-determining regions (CDRs) are hypervariable regions determining specificity.

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During V(D)J recombination, the variable region of the TCR chains, which is the binding site determining specify towards the MHC-peptide complex, is re-organized starting with TCRβ and later TCRα. The various variable (V), diversity (D) and joining (J) genes included in the genome are randomly joined to create unique TCR. Further increasing the diversity of the resulting re-arranged genes is the terminal deoxynucleotidyl transferase (TdT) that introduces randomly additional nucleotides when reattaching the DNA strains [65, 66].

The recombination-activating genes (RAG)-1 and -2 are essential enzymes for V(D)J recombination [67, 68]. A similar process also dependent on RAG molecules occurs in B cells with their B cell receptor (BCR). Hence, mice deficient in RAG lack both T cells, B cells, and any other non-classical cells also requiring re-arrangement, such as NKT cells and γδT cells [69, 70].

1.3.4 TCR signaling

The TCR is composed of a variable region responsible for binding the MHC-peptide complex, and a constant region associated to signaling molecules [71-73]. The TCR interacts with the MHC-peptide complex, while the co-receptors CD4 or CD8 and adhesion molecules help at the formation of a stable interaction. Upon binding, the co-receptors help recruiting the tyrosine kinase Lck which phosphorylates ITAMs motives of CD3 chains [74]. This

phosphorylation allows the recruitment and phosphorylation by Lck of the zeta-chain associated protein kinase 70kDa (ZAP70) [75]. ZAP70 then phosphorylates key residues of the linker for activation of T cells (LAT), leading to LAT signalosome formation and subsequent signal transduction by three branches: Ca^{2+} influx, MAPK and NF-κB (Figure 5) [71].

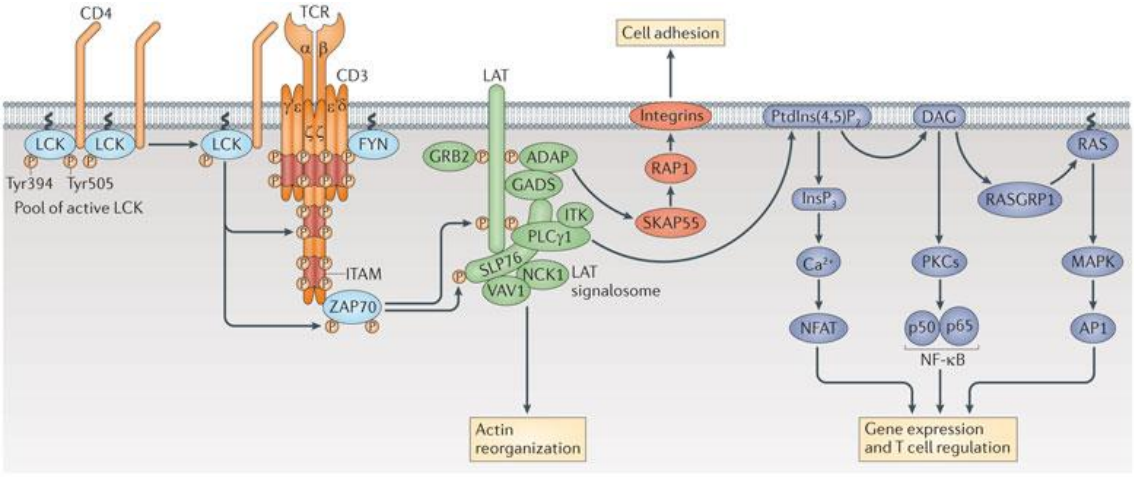

Figure 5 – TCR signaling pathways

Phosphorylation events following recognition of the MHC-peptide complex by the TCR leading to T cell activation. Phosphorylation of ITAM motives on CD3 favors the recruitment of ZAP70 that then phosphorylates LAT, leading to subsequent intracellular signaling.

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1.3.4.1 ZAP70

ZAP70 is a tyrosine kinase that has an essential role in signal transduction through the TCR signalosome. The association of ZAP70 to CD3 ζ-chains is a crucial step in T cell signaling, and happens within seconds of antigen recognition by the TCR [76]. Whereas thymocytes in early stages of development also express spleen tyrosine kinase (Syk) for signal transduction, mature T cells solely rely on ZAP70, making it a central protein in T cell activation [77]. Loss of function in human leads to severe combined immunodeficiency characterized by the absence of peripheral $CD8^+$ T cells, and normal number of nonfunctional $CD4^+$ T cells [78, 79]. In mice however, thymocytes development is completely blocked at the double positive (DP) stage in $ZAP70^{-/-}$, resulting in an absence of both $CD4^+$ and $CD8^+$ T cells in the periphery [80]. This distinction might be explained by a different expression of Syk and ZAP70 during the development of $CD4^+$ T cells. Nevertheless, the lack of function of $CD4^+$ T cells in human demonstrates the essential role of ZAP70 in mature T cells.

Although it is usually associated to T cell activity due to this non-redundant role, other immune cells also express ZAP70. In these cells, ZAP70 is also responsible for signal transduction of membrane receptors bearing ITAM motives (e.g. FcRγ, BCR, and killer cell-activating receptor-associated protein (KARAP)) [20, 81]. It was described in mice and human B cells depending on cellular maturity, and in human B cells malignancies such as chronic lymphocytic leukemia [82]. Expression of ZAP70 in B cells is associated to increased BCR signaling [83]. However, unlike in mature T cells, B cells signal transduction relies mostly on Syk [84]. On the other hand, NK cells rely on both Syk and ZAP70, with noticeable reduction in activity only in double knockout mice, in which cytotoxicity is not even completely abolished due to several redundant activation pathways [81, 85].

1.3.5 T cell selection

The great advantage of the random re-arrangement of TCR is that it allows recognition of a vast library of antigens without a need for each specific TCR to be included in the genome. This library is so vast that virtually any peptides presented by an MHC molecule could be recognized by a specific TCR. However, this also leads to the generation of both non-functional and self-reactive T cells. Whereas the organism would waste resources keeping non-functional T cells, self-reactive T cells are potentially harmful. The purpose of T cell selection is to keep only T cells expressing a functional TCR but that do not react against self-antigens. These selections processes are respectively called positive and negative selections [80, 86].

Selections occur in the thymus, in which T cells follow several steps of maturation (Figure 6) [87, 88]. Lymphoid progenitors enter the cortex of the thymus when they are at the double negative 1 (DN1) stage, meaning that they are negative for both CD4 and CD8, and are $CD44^{+}CD25^{neg}$. The β-chain is first rearrange, and from DN3 to DN4, successful pre-TCR signaling is required for survival. At this stage, thymocytes rely on both ZAP70 and Syk for signal transduction. At the DP stage, thymocytes express both CD4 and CD8, and have now re-arranged both the α- and the β-chains. Positive and negative selections then occur in the medulla. Positive selection is the requirement of successful TCR signaling for survival following recognition of self-MHC [89]. Negative selection is the removal of clones highly reactive to self-antigens, a process dependent on the presentation of self-antigens in the thymus by APC such as mTEC [90, 91]. Ectopic expression of tissue-restricted self-antigens in the thymus by various mechanisms, including a critical role of AIRE, is essential to central tolerance [92]. Both positive and negative selections are dependent on TCR signaling strength, which is proportional to the affinity between the MHC-peptide complex and the TCR. At this stage, TCR signaling relies solely on ZAP70 since thymocytes lost expression of Syk [77].

1.3.6 T cell activation

Naïve T cells, which have never encountered their antigen, circulate in the blood and travel to various lymph nodes where APC constantly present antigens. TCR recognition of the

MHC-peptide complex is the first step towards activation. CD4 or CD8 serve as co-receptor, binding either MHC-II or MHC-I respectively, and help to recruit Lck which is needed for TCR signaling [93, 94]. A second signal is however essential, otherwise T cells become anergic. This secondary signal, referred to as co-stimulation, relies on the expression of co-stimulatory molecules by professional APC [95]. The most carefully characterized are the activating CD80-CD86/CD28 and the inhibitory PD-1/PD-L1, however many others have also been described [96]. Inhibitory molecules have drawn a lot of attention due to their involvement in cancer immunoevasion. These findings led to the recent approval of checkpoint inhibitors for cancer immunotherapy [97]. Finally, the cytokines microenvironment, which is considered by some immunologists as a third signal, tailors T cells differentiation [98, 99].

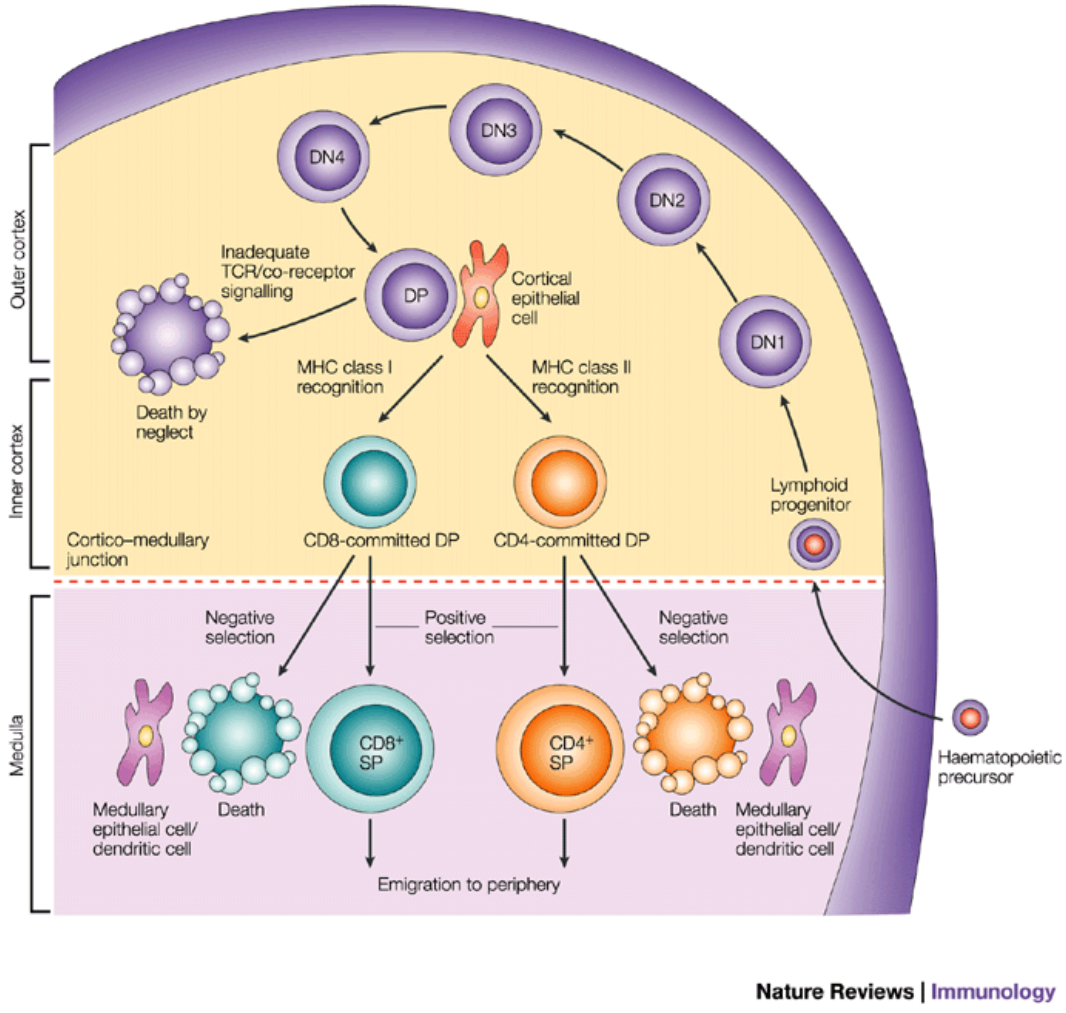

Figure 6 – T cell development in the thymus

The various steps of maturation of thymocytes from lymphoid progenitors to mature SP T cells, including positive and negative selections.

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The organization of the various binding molecules during APC/T cell interaction is referred to as the immunological synapse [100-102]. Organization of receptors in lipid rafts is important for its formation [103]. Other than signaling molecules, the immunological synapse also includes adhesion molecules (e.g. ICAM-1, LFA-1) stabilizing the interaction between both cells.

1.3.7 Effector functions of T cells

Two subsets of αβT cells can be distinguished based on their expression of CD4 or CD8 co-receptors. Whereas $CD8^+$ T cells recognize intracellular peptides presented on MHC-I, $CD4^+$ T cells recognize extracellular peptides presented on MHC-II [2]. This distinction is important to understand the difference in their functions.

1.3.7.1 Cytotoxic T lymphocytes (CTL)

$CD8^+$ T cells, also called cytotoxic T lymphocytes (CTL), induce apoptosis of the presenting cell. Once activated CTL detect their antigen on a cell, they release granules containing perforin and granzymes, leading to membrane permeation and apoptosis of the targeted cell [1]. CTL also induce apoptosis through Fas/FasL interaction, as well as TRAIL/TRAIL-R. Since all nucleated cells express MHC-I and constantly present cytosolic antigens, this mechanism insures that compromised cells, either from infection or malignancy, are eliminated promptly to contain the risk.

1.3.7.2 T helper lymphocytes (Th)

On the other hand, $CD4^+$ T cells recognize extracellular antigens presented on MHC-II, meaning that the presenting cell is not necessarily compromised, but rather the surrounding environment. The role of $CD4^+$ T cells, referred to as T helper cells (Th), is to orchestrate an immune response appropriate to the pathogen itself. To do so, Th secrete at the site of inflammation chemokines to attract the appropriate cells, and cytokines to elicit cellular response. Cytokines secreted by innate cells in response to DAMPs/PAMPs will fine-tune the differentiation of Th in different subsets, which in turns secrete a different set of cytokines. Common nomenclature divides Th into Th1, Th2, Th17 and regulatory T cell (Treg) (Figure 7) [104-106].

Activation of the transcription factor T-bet following exposure to IL-12 and IFN-γ leads to differentiation into Th1 [107]. A Th1 response is characterized by the secretion of IFN-γ and IL-2, which activate macrophages and favor phagocytosis of pathogens. This response is particularly important against intracellular pathogens, but it is also considered pro-inflammatory and would be associated to autoimmune diseases [108].

Th2 differentiation is under the control of the transcription factor GATA-3, which is activated in response to IL-4 [109]. Th2 secrete IL-4 and IL-5 to induce B cells proliferation and help humoral response [110]. These Th2 cytokines are associated to parasite infections, such as

helminths, and are implicated in allergic reaction and asthma [111-113]. Th2 cytokines also exert anti-inflammatory and wound healing effects.

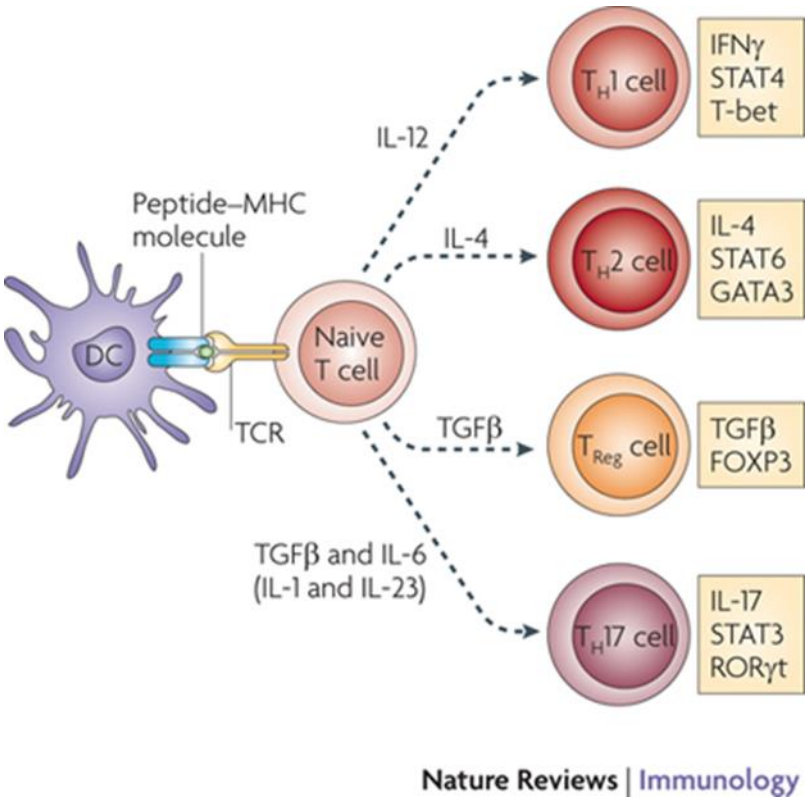

Figure 7 – Differentiation of CD4$^+$ T cells in various subsets of effector cells

Following activation by APC, the cytokines microenvironment influences the differentiation of CD4$^+$ T cells in 4 subsets of effector cells.

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More recently, a subset of Th producing IL-17 was identified [114, 115]. This finding totally changed the Th1/Th2 paradigm [116]. Their implication in autoimmunity has also steered a debate regarding the implication of either Th1 or Th17 in these diseases [117]. Other than IL-17, their production of GM-CSF is also contributing to autoimmunity [118]. Th17 differentiation is under the control of the transcription factor ROR-γt, which is activated in mice by TGF-β and IL-6 [119]. Although it does not seem to affect directly the commitment to Th17 lineage, IL-23 has an important role in the maintenance of Th17 [120]. Th17 are implicated in the clearance of extracellular infections such as fungi by the recruitment of inflammatory cells like neutrophils [121, 122].

Finally, a subset of CD4$^+$ T cells differentiate into Treg following the induction of the transcription factor Foxp3 [123, 124]. These cells have immunosuppressive functions. They can either originate from thymic development (natural Treg, nTreg) or be induced by

cytokines such as TGF-β in the periphery (induced Treg, iTreg) [125]. Regarding immunosuppressive mechanisms, Treg are known to secrete IL-10 and TGF-β, which could explain immunomodulation from soluble factors. Treg also express high level of CD25, the high affinity receptor for IL-2. Another regulatory mechanism involves the critical role of CTLA-4 [126, 127]. Finally, it is worth mentioning that TCR signaling is needed by Treg for proper immunosuppressive activity [128].

2 AUTOIMMUNE DISEASES

Autoimmune diseases are a family of diseases which share a similar pathogenesis; the activation of the immune system against self-antigens. Classification of common autoimmune diseases traditionally focused on affected organs: psoriasis (skin), rheumatoid arthritis (joints), type I diabetes (beta islets of the pancreas), multiple sclerosis (central nervous system), Crohn's disease (gastrointestinal tract) and ulcerative colitis (distal colon) [129-133].

2.1 PSORIASIS

Psoriasis is an autoimmune disease of the skin with a prevalence of approximately 2% [134]. Like many other autoimmune diseases, both genetic predispositions and environmental factors contribute to its onset [135-137]. It is characterized by erythematous plaques covered by white scales. Psoriatic lesions are characterized by hyperplasia and incomplete differentiation of keratinocytes in the epidermis, accumulation of leukocytes in the skin and increased angiogenesis [130]. Approximately one third of patients also suffer from associated joints inflammation, a disease called psoriatic arthritis [138, 139].

2.1.1 Epidemiology of psoriasis

Epidemiologic studies reveal that prevalence varies between ethnic groups, with higher prevalence among Caucasians, and lower prevalence among Japanese, aboriginal from Australia and South America [140, 141]. The first symptoms can appear at different ages. However, two peaks of onset are described; early onset between 20 and 30 years, and late onset between 50 and 60 years [142, 143].

2.1.2 Genetics of psoriasis

The contribution of genetic predispositions to psoriasis susceptibility has long been known due to the higher prevalence within the same family [144, 145]. Studies in twins indicate an increased prevalence in monozygotic twins, demonstrating the importance of genetics. However, the concordance of only 30% demonstrates the major role of environmental factors in psoriasis onset [146, 147]. Nowadays, large genome-wide association studies have identified 36 risk loci [135, 148]. Most genes are implicated in the immune response, but some are also associated to barrier development of the skin [149].

The strongest association is found within *HLA-C*, which suggests a role for T cells in this pathology [150, 151]. Other major risk loci associated to T cells include *IL12B* and *IL23R* [152, 153]. The implication of these cytokines suggests biased differentiation of Th to pro-inflammatory phenotype through the IL-23/Th17 axis [154, 155]. Finally, it is interesting to note that polymorphism in the protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*) was associated to early onset psoriasis, a gene that is also strongly associated to other autoimmune diseases, including RA [156, 157].

2.1.3 Disease mechanisms of psoriasis

There are three main components in the pathogenesis of psoriasis: epidermis alterations, activation of the immune system, and angiogenesis [130]. All three components interact closely, for instance increased angiogenesis and leaky blood vessels contribute to the accumulation of leukocytes. These leukocytes secrete cytokines, which affect growth and differentiation of keratinocytes within the skin, leading to hyperproliferation and chemokines secretion which further increase recruitment of leukocytes and angiogenesis [158]. Figure 8 illustrates mechanisms proposed by Nestle et al. 2009 of initiation and maintenance of psoriatic lesions [134].

2.1.3.1 Epidermis

Hyperplasia of keratinocytes in the epidermis is characteristic of psoriatic lesions. The basal cells divide on average every 40 hours in psoriatic lesions, instead of 200 hours in healthy skin [159]. This rapid turnover leads to incomplete differentiation and maturation of keratinocytes, affecting the integrity of the skin [160]. Keratinocytes normally lose their nucleus during differentiation in the *stratum corneum* of healthy skin, however in psoriatic lesions, nucleuses are often observed, a phenomenon called parakeratosis that is indicative of incomplete differentiation [161]. This incomplete differentiation is also reflected by the keratin produced in psoriatic lesions, which are mostly K6/16 and K17 rather than K1/10 normally associated to terminally differentiated keratinocytes [162, 163].

2.1.3.2 Immune response

As mentioned previously, genetic predispositions strongly support the implication of the immune system and the IL-23/Th17 axis [164, 165]. Numerous animal models and therapies also support a role for T cells [166, 167]. Moreover, recent drug development has focused on controlling aberrant T cells activity in psoriatic lesions [168]. For example, cyclosporine A, which is an immunosuppressive drug inhibiting calcineurin and thus reducing the production of IL-2, is used to treat severe psoriasis [169]. More recently, drugs directly targeting the IL-17/IL-23 pathway were approved. Other immune cells also have a role in the development of skin lesions, including cells of the innate immunity. For example, it was demonstrated that neutrophils were essential in the flaky skin mice model to develop skin lesions [170]. Moreover, a case report in human describes the resolution of psoriasis during agranulocytosis in a patient receiving ticlopidine, and the later relapse of skin lesions upon normalization of circulating neutrophils [171]. The importance of the IL-17/IL-23 pathway corroborates with an implication of neutrophils in psoriasis development.

2.1.3.3 Angiogenesis

The importance given to angiogenesis in the pathogenesis of psoriasis has increased in the past few years, some authors even suggesting it to be a driving factor rather than a consequence of skin inflammation [172]. Most of the approved drugs to treat psoriasis affect either directly or indirectly blood vessels [173]. The excessive angiogenesis is the result of

increased local concentrations of vascular endothelial growth factor (VEGF) [174-176]. Interestingly, transgenic mice expressing constitutively VEGF in the basal layer of the epidermis had both increased angiogenesis, and also increased adherence and recruitment of leukocytes in the skin [177].

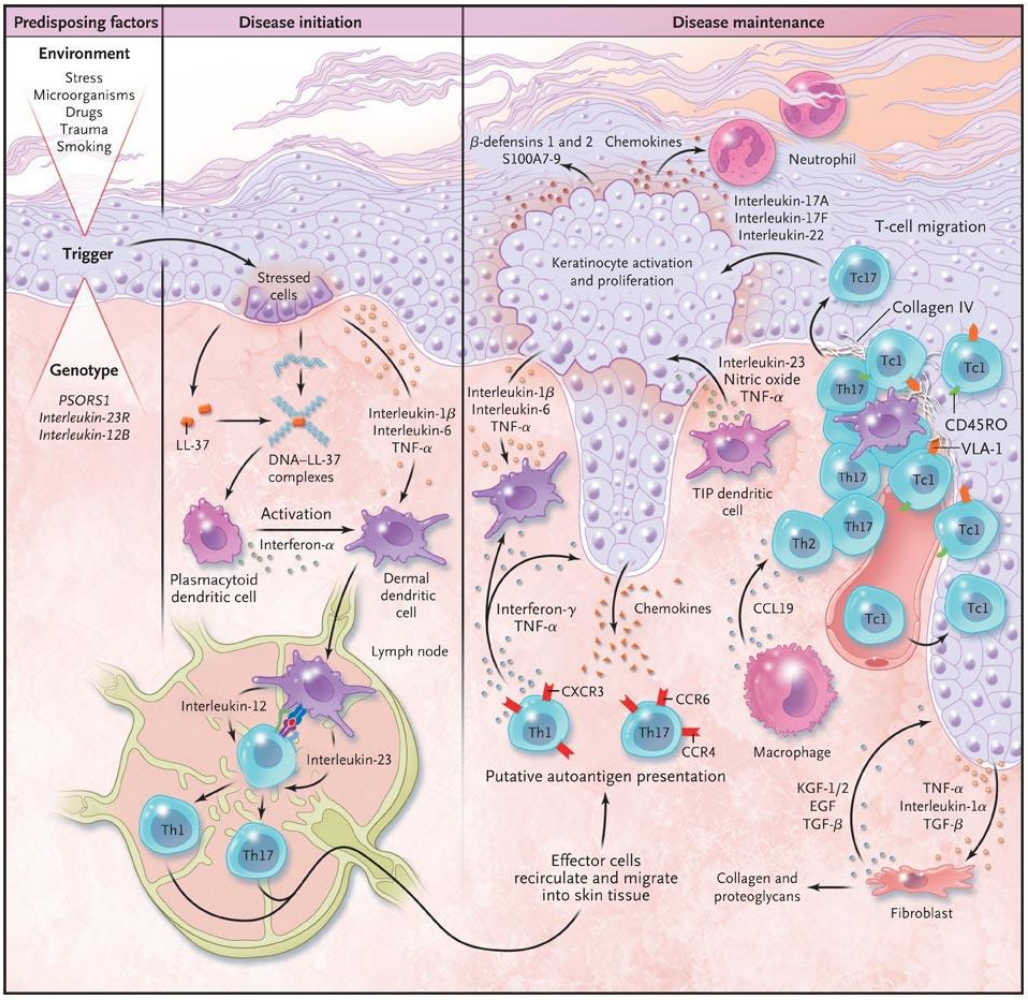

Figure 8 – Mechanisms of psoriasis from initiation to maintenance

Schematic representation of the evolution of psoriatic lesions proposed by Nestle et al. 2009 [134]. In individual genetically predisposed to psoriasis, environmental factors will trigger activation of innate immunity in the skin. APC then migrate to dLN where activation of Th1 and Th17 occur. Recruitment of activated T cells in lesions and secretion of cytokines perpetuated the activation and hyperproliferation of keratinocytes, and the expansion of blood vessels.

2.1.4 Treatments of psoriasis

Although lesions can be treated, there is currently no cure for psoriasis [178]. Patients will therefore experience remissions and relapses throughout their life, significantly decreasing their quality of life [179, 180]. A complete description of psoriasis treatments and clinical guidelines is outside the scope of this thesis; however it is worth mentioning that most novel therapies focus on controlling the immune system rather than controlling directly the proliferation of keratinocytes. The use of biologic drugs in the past two decades has drastically improved the treatment of patients suffering from severe psoriasis. The most commonly used biologic drugs are TNF-α blockers, which have now been used for almost two decades [181, 182]. More recently, two other monoclonal antibodies targeting IL-12/23 (ustekinumab) and IL-17 (secukinumab) were approved by the FDA for the treatment of severe psoriasis, highlighting once again the importance of Th17 in the pathogenesis of psoriasis [134, 183, 184].

2.2 RHEUMATOID ARTHRITIS (RA)

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation and progressive destruction of joints. It affects approximately 1% of the worldwide population [131]. RA is not only reducing life quality of patients: it is also the leading cause of disability in the US, representing a significant societal burden through both high direct (e.g. treatments) and indirect costs (e.g. loss of productivity) estimated at $128 billion in 2003 [185-187]. Its exact pathogenesis remains unknown, however genome-wide association studies have now identified more than 100 risk loci, and several environmental factors have been associated to RA [188-190]. A better understanding of the immunopathogenesis of RA has helped improve drastically treatments over the past two decades. Animal models of arthritis have been central to these developments, and remain essential to discover and investigate novel therapeutic targets.

2.2.1 Diagnosis

In 2010, the American College of Rheumatology and the European League Against Rheumatism published a new guideline to help clinicians diagnose RA [191]. Table I presents their scoring system based on the number of affected joints, the duration of symptoms, and serologic markers. A diagnosis of RA is defined as a score of 6 points or more. Interestingly, this updated guideline introduced antibodies to citrullinated protein antigens (ACPAs) as diagnosis criteria of RA. Extensive research is still conducted to further identify biomarkers for diagnosis and prognosis of RA.

2.2.2 Antibodies to citrullinated protein antigens (ACPAs)

The discovery and association of ACPAs to RA has been a major breakthrough in the field. This association had been described long before the publication of the 2010 guideline [192, 193]. Interestingly, APCAs can be detected many years before the apparition of symptoms [194, 195]. They also have a prognosis value, being associated to more severe radiologic

damage in the joints [196, 197]. The direct pathogenicity of these antibodies remains unclear and somewhat controversial, some groups claiming that they either enhance or even induce arthritis in rodents, whereas others could not reproduce these findings [198-200]. Nevertheless, ACPAs association to RA is undeniable, and suggests immunological abnormalities long before clinical onset.

Table I - The ACR/EULAR 2010 classification for RA

Criteria	Score
A. Joint involvement:	
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without large joints involved)	2
4-10 small joints (with or without large joints involved)	3
>10 joints (at least 1 small joint)	5
B. Serology (at least 1 test result is needed for classification as RA):	
Negative RF and negative anti-CPP antibodies	0
Low-positive RF or low-positive anti-CPP antibodies (≤ x3 upper normal limit)	2
High RF or high anti-CPP antibodies (> x3 upper normal limit)	3
C. Acute phase reactants:	
Normal CRP level and normal ESR	0
Abnormal CRP level or abnormal ESR	1
D. Duration of symptoms:	
< 6 weeks	0
≥ 6 weeks	1

Note: To be applied only to patients: (1) who have at least 1 joint with definite synovitis, excluding the DIP joints, first MTP joints, and first CMC joints, and (2) in whom the synovitis cannot be explained by another disease. Definite RA is defined as a cumulative score of 6 points or more (max 10).

It is often suggested that RA should be divided in $ACPA^{+}$ and $ACPA^{neg}$, a distinction of importance in genetic studies since the risk factors associated to those two subtypes are likely to differ significantly [201-203]. Reclassification of diseases based on biomarkers and pathogenesis, rather than anatomy and symptoms, is a common trend in modern medical research, in particular in oncology (e.g. $HER2^{+}$ breast cancer) because it affects therapeutic strategies. The discovery of ACPAs has not only helped in the diagnosis and prognosis of RA, but it has also helped researchers to investigate novel disease mechanisms leading to several interesting hypotheses explaining various genetic and environmental risk factors.

2.2.3 Epidemiology of RA

Prevalence of RA varies geographically; higher prevalence being observed in Northern Europe and North America compared to South America, Asia and Africa [204]. The prevalence is particularly high among Native Americans, reaching up to 7% in certain groups [205]. Women are 2 to 3 times more likely than men to have RA [204, 206]. The first symptoms of RA can appear at various ages, with a peak of incidence at around 50 years old [207]. It is worth mentioning again that immunological changes, such as production of self-reactive antibodies, can precede the diagnosis of RA by up to 10 years [194, 195, 208, 209].

2.2.4 Genetics of RA

Genetic predispositions have an important role in the development of RA. Family history is a major risk factor to develop RA, with a calculated heritability of 65% [210]. Studies in twins revealed a concordance of approximately 15% and 4% in monozygotic and dizygotic twins respectively, a clear increase compared to baseline prevalence. Still, the low concordance highlights the importance of gene-environment interactions to trigger the disease [211, 212]. It also suggests that prevention is possible since genes by themselves are not sufficient.

More than 100 risk loci have now been identified by genome-wide association studies [188, 189, 213]. Most loci identified are believed to affect the immune system, and are frequently associated to T cells activity. Considering the high number of risk loci and their relatively low contribution to RA (e.g. OR 1.05), this section will focus on the two most important loci, which are also directly relevant to the work presented in this thesis.

2.2.4.1 HLA-DRB1

By far, the most important genetic contribution is within the MHC region, which contributes for more than all other risk loci identified taken altogether [189]. Several susceptibility alleles have been identified, the most characterized within the *HLA-DRB1*04* group [214]. The shared epitope hypothesis claims that the amino acid sequence 70-74 from the HLA-DRβ chain is critical at conferring arthritis susceptibility [215, 216]. It is believed that the shared epitope is crucial in self-antigen presentation. Interestingly, an association between *HLA-DRB1* and antibody response, in particular anti–cyclic citrullinated peptides (CPP) antibodies, has been found [217-219]. Major efforts to understand this link between antigen

presentation from disease promoting HLA alleles, posttranslational modifications and antibodies production have been made in the past few years [199, 220]. Studies have also found that disease progression is more severe in patients with both anti-CPP antibodies and a shared epitope allele [214, 219].

2.2.4.2 PTPN22

The strongest non-MHC genetic association is within *PTPN22*, a protein tyrosine phosphatase expressed primarily in lymphoid cells [221, 222]. In T cells, PTPN22 inhibits TCR signaling by dephosphorylating various kinases of the Src (e.g. Lck) and Syk families (e.g. ZAP70) [223]. Its activity relies on binding with Csk [71]. The risk allele in *PTPN22* (*1858C>T*) leads to a R620W substitution in the protein in one of the four proline-rich SH3 binding sites, and was found to affect binding to Csk [223, 224]. The mutation causes a gain-of-function of PTPN22, and thus a reduction of TCR signaling [225, 226]. An interesting hypothesis regarding the mechanism of autoimmunity is that this reduction in TCR signaling would affect central tolerance in the thymus, leading to self-reactive T cells escaping deletion [222, 227]. Another hypothesis is that it affects the removal of self-reactive B cells [228-230].

The first causative hypothesis linking the mutation to autoimmunity is particularly relevant to this thesis due its parallel with the SKG model of arthritis. Apart from RA, this mutation is also strongly associated to other autoimmune diseases such as type I diabetes, systemic lupus erythematous, and Grave's disease [231-234]. The association of this allele with various autoimmune diseases is in accordance with an unspecific mechanism of break of tolerance such as the defective thymic selection hypothesis or the B cells hypothesis.

2.2.5 Environmental risk factors of RA

As with most autoimmune diseases, RA is a complex multifactorial disease in which both genes and environmental factors contribute to disease onset. A common view is that environmental factors can trigger a chain of events leading to RA, but only in individuals with particular genetic predispositions.

2.2.5.1 Smoking

Smoking has long been suggested as an environmental risk factor to RA based on epidemiologic studies [235-238]. Interestingly, a study in discordant twins also supports this association, however the sample size was relatively small [239]. Data also suggest that smoking could affect the severity of RA, such as increased radiographic progression of joints damage, and the development of rheumatoid factor (RF) [240-242]. However at that time, the underlying mechanism was unclear.

More recently, advances in the understanding of the genetic contribution of RA, and the discovery of ACPAs, have shed lights on a potential mechanism. First, it was observed that smoking increases the risk of developing anti-CPP antibodies in patients with the HLA-DRB1 shared epitope [243, 244]. Interestingly, smoking increases the expression of

peptidylarginine deiminase 2 (PAD2) in the lungs, thus increasing protein citrullination [245]. Further experiments confirmed the association of smoking, the presence of HLA-DRB1 shared epitope and ACPAs, suggesting that smoking promotes non-specific citrullination of proteins, supporting this lung hypothesis [246]. This is a particularly elegant gene-environment association which requires further investigations [247]. However, this association could not be replicated in an independent study using different cohorts in North America [248]. Some studies also suggest that environmental risk factors such as smoking would be more important than genetic predispositions at developing ACPAs, whereas genetic predispositions would be more important in determining which $ACPA^+$ individuals ultimately develop RA [249].

2.2.5.2 Sex hormones

Sexual hormones are also thought to influence arthritis. This is exemplified by the higher prevalence in women compared to men. Pregnancy is known to improve symptoms and have an impact on the time of disease onset [250-252]. Moreover, epidemiologic studies suggest that oral contraceptives could improve the clinical outcome of RA [236, 253-255]. The suppressive effect of female sex hormones was also clearly demonstrated in rodent models, including in collagen induced arthritis (CIA) [256-258]. Hormone replacement therapy in RA patients was shown to have a modest effect on disease progression [259, 260]. However, this protective effect is not believed to be specific for RA. Instead, estrogen would have an immunosuppressive role which is more general [261, 262]. One study for example suggested that it would drive the expansion of Treg [263]. The modest benefit observed in RA is however not sufficient to justify the use of hormone replacement therapy considering the potential increased risk of cancers [264].

2.2.5.3 Bacteria

As with other autoimmune diseases, infections and peptide mimicry were believed to have a significant role in RA [265, 266]. Animal studies suggested that some infectious agents could trigger and exacerbate arthritis [267]. Despite major interest in this hypothesis due to association studies, causative evidence in human is limited [268]. Hence, this theory was put aside for some time.

More recently, a very interesting mechanism was proposed to link bacteria and RA. Once again, the association between ACPAs and RA has been central. Epidemiologic studies revealed that RA patients have an increased risk of periodontitis [269]. Periodontitis in adults is usually caused by *Porphyromonas gingivalis*, a bacterium expressing a PAD enzyme which could citrullinate host proteins [270]. Antibodies against *Porphyromonas gingivalis* were indeed found to be associated with an increased risk of ACPAs in RA patients [271, 272]. This is a very elegant theory linking environmental risk factors to a potential pathological mechanism.

2.2.6 Treatment of RA

The following section is not meant to cover in details therapeutic strategies for RA. Instead, it focuses on major breakthroughs which provided valuable information on the physiopathology of RA: clinical efficacy in patients being the ultimate proof-of-concept that a particular pathway is important. Understanding pathways implicated in human RA helps to evaluate the relevance of various animal models of arthritis. Clinical guidelines would also take into consideration potential side effects and costs; however both of these criteria provide little insight on the disease itself and are thus not directly relevant to this thesis. The American College of Rheumatology published recently their 2015 guidelines [273].

2.2.6.1 Glucocorticoids

The broad anti-inflammatory action of glucocorticoids is effective to control symptoms of RA and is still considered a valid temporary addition in initial therapy of moderate to severe RA. It can also be used to treat flares. However, it should be used to the lowest possible dose and for the shortest possible duration considering the serious side effects associated to glucocorticoids.

2.2.6.2 Disease-modifying antirheumatic drugs (DMARDs)

As opposed to glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g. naproxen, indomethacin, diclofenac, ibuprofen) which help to control pain and inflammation rapidly but do not affect RA progression, disease-modifying antirheumatic drugs (DMARDs) usually act slowly but improve the evolution of RA. Hence, their use is crucial for the long term management of RA. DMARDs are now divided in traditional/chemical drugs, and biologic drugs. Despite major advances in biological treatments, traditional DMARDs are still considered a cornerstone of initial therapy of RA. These drugs include methotrexate, sulfasalazine, leflunomide, hydroxychloroquine and gold salts.

A low dose of methotrexate is the most commonly used DMARD therapy. It is an antimetabolite and antifolate drug. It has several effects on the immune system which could explain efficacy in RA, including inhibition of T cells and B cells [274, 275]. Table II presents biologic DMARDs approved for the treatment of RA.

2.2.6.3 TNF-α blockers

The introduction of TNF-α blockers revolutionized treatment of RA almost two decades ago. These drugs target the pro-inflammatory cytokine TNF-α and have a very good efficacy with a fairly favorable side effects profile. Even nowadays, they are considered the gold standard of RA therapy. Among the list of the top 10 global sales for a drug in 2015, 3 are TNF-α blockers (Humira, Remicade and Enbrel). TNF-α blockers are usually used in patients after unsuccessful initial treatment with traditional DMARD, in particular methotrexate. However, this use as second line therapy is mostly explained by the high cost of biologic drugs such as TNF-α blockers [276]. Hence, this recommendation is mostly based on pharmacoeconomics rather than pharmacotherapy. Upon failure with methotrexate, monotherapy with TNF-α

blockers is considered, or combination with methotrexate in partial responders since there is a demonstrated benefit of the combination [277].

Table II - Biologic drugs in the treatment of RA

Drug Name	Brand	Description	Approval	Other indications
Infliximab	Remicade	Chimeric (mouse/human) anti-TNF-α	1998	Psoriasis, ulcerative colitis, Crohn's disease
Etanercept	Enbrel	TNF receptor/Fc fusion protein	1998	Psoriasis
Anakinra	Kineret	IL-1R antagonist	2001	-
Adalimumab	Humira	Human IgG1 anti-TNF-α	2002	Psoriasis, ulcerative colitis, Crohn's disease
Abatacept	Orencia	CTLA-4/Fc fusion protein	2005	-
Rituximab	Rituxan	Chimeric (mouse/human) anti-CD20	2006	-
Certolizumab	Cimzia	PEGylated Fab' anti-TNF-α	2008	Psoriatic arthritis, Crohn's disease
Golimumab	Simponi	Human IgG1κ anti-TNF-α	2009	Psoriatic arthritis, ulcerative colitis
Tocilizumab	Actemra	Human IgG1 anti-IL-6	2010	-

Approval: year of approval by the FDA for the treatment of RA
Other indications: other FDA indications in autoimmune diseases relevant for this thesis

The success of TNF-α blockers has obviously generated a large amount of research, both on TNF-α but also other cytokines [278]. Although some patients do not respond to these treatments, others experience total remission. This is due to the unique role of TNF-α in the inflammatory cascade. In fact, TNF-α blockage is useful for several other autoimmune diseases, including psoriasis and Crohn's disease, demonstrating how central TNF-α is in autoimmunity [181, 279]. Not so surprisingly, an increased risk of severe infections and a potential association to malignancies are the biggest concerns with these treatments [277, 280]. The association to malignancies is however still debated [281, 282].

2.2.6.4 CTLA4-Ig

On T cells, CTLA-4 is an inhibitory receptor binding B7 proteins (CD80 and CD86) of APC. Abatacept is a fusion protein composed of the extracellular domain of CTLA-4 and the Fc region of IgG1. It acts by binding B7 co-stimulatory molecules on APC, thus preventing interaction between B7 and CD28, and therefore T cells activation [283]. It is indicated for the treatment of RA refractory to TNF-α blockers [284]. Some data suggest favorable comparison to TNF-α blockers, however the large amount of safety information with TNF-α blockers along with extensive clinical experience help them maintain their status as a first choice within biologic DMARDs [285]. It is interesting to note that blockage of CTLA-4 is currently used in cancer immunotherapy to increase T cells response towards malignant cells [97].

2.2.6.5 Anti-IL-6R

Tocilizumab, a monoclonal antibody against IL-6R, is also approved for the treatment of RA in combination with methotrexate [286]. The development of anti-IL-6R therapy originates from the observation that IL-6 is overexpressed in RA [287, 288]. The biological function of IL-6 was fairly difficult to understand since it is produced by a wide variety of cells and also exerts its effects in various cell types [289]. It is now clear that it is associated to the Th17 pathway [290, 291]. Tocilizumab was shown to improve clinical outcome in patients with poor response to TNF-α blockers [289]. Similarly to TNF-α, IL-6 was associated to a variety of autoimmune diseases in animal models, suggesting a central and non-redundant role in chronic inflammation [292, 293].

2.2.6.6 IL-1RA

Anakinra is a recombinant IL-1 receptor antagonist (IL-1RA) approved in RA. It acts by inhibiting the action of IL-1 by preventing its binding to the IL-1R. Secreted by cells of the innate immunity, IL-1 has pro-inflammatory properties [294]. Anakinra improves clinical outcome in patients receiving methotrexate [295]. This treatment generated a lot of interest when it was found to block bone resorption [296, 297]. However, although direct comparison are not available, meta-analyses suggest that Anakinra is less effective than TNF-α blockers, making this treatment less appealing to clinicians [298, 299].

2.2.6.7 Anti-CD20

Rituximab is a monoclonal antibody recognizing CD20, a protein expressed by B cells. It acts by depleting B cells which are responsible for humoral response and are also potent APC. Rituximab was demonstrated to improve clinical outcome in patients receiving methotrexate, including patients refractory to TNF-α blockers [300, 301]. Although it is officially indicated for the treatment of RA, the main indication of rituximab is in B cell malignancies [302, 303]. B cells depletion has also been investigated for other autoimmune diseases including multiple sclerosis and systemic lupus erythematosus [304-306].

2.3 ANIMAL MODELS OF ARTHRITIS

The complex interplay between genetic and environmental factors in RA makes mechanistic studies in human challenging. Several rodent models that mimic various aspects of human RA have been developed in order to dissect the pathophysiological mechanisms and evaluate novel therapeutic targets [307, 308]. Mice models are particularly useful for early investigations to complement *in vitro* experiments due to the available technology in terms of genetic manipulations, as well as their relatively low cost and short gestation period.

The most commonly used model of arthritis is collagen induced arthritis (CIA), a model based on the induction of arthritis by immunization with the joint-specific protein collagen type II (CII) and an adjuvant, typically complete freund's adjuvant [309]. Other common models include collagen antibody-induced arthritis (CAIA) and adjuvant-induced models such as pristane induced arthritis (PIA) in rats [310]. In addition to these inducible models, other models are based on genetic modifications that increase susceptibility for arthritis, among which the SKG model that shares several interesting characteristics with human RA.

2.3.1 Collagen induced arthritis (CIA)

CIA is the gold standard among animal models of arthritis [311, 312]. Studies using CIA in mice have allowed the identification of several disease promoting polymorphisms. Similarly to RA, MHC haplotype is a major genetic factor in CIA. As mentioned above, the model in mice relies on immunization with CII, usually from heterologous origin. Proper presentation of the T cell epitope by CII-permissive MHC (e.g. H2-A^q and H2-A^r) is essential to confer susceptibility [309, 313]. In agreement with this finding, αβT cells, but not γδ T cells, were found to be essential in CIA [314].

The immunodominant T epitope in mice expressing H2-A^q is the galactosylated $CII_{260\text{-}270}$ peptide [315]. Post-translational galactosylation of the K264 amino acid in the cartilage is thought to be a critical step in CIA [316, 317]. This peripheral modification of CII could explain why T cells reactive to this modified peptide are not negatively selected in the thymus since it appears not to be presented by mTEC. This is an elegant explanation to the break of central tolerance of T cells. It is also particularly relevant to human RA due to the parallels with peripheral citrullination of proteins.

The break of T cells tolerance ultimately leads to the production of high levels of anti-CII antibodies in sera [318]. Unlike with T cells, B cells (and antibodies) are highly cross-reactive to self-CII due to homology in B cells epitopes. B cell deficient mice do not develop CIA. On the other hand, serum transfer, which contains self-reactive antibodies, is pathogenic, demonstrating the essential role of this humoral response [319, 320]. This is quite interesting when considering the efficacy of rituximab in RA. Extensive characterization of B cells epitopes led to the development of another model of arthritis: CAIA (see section 2.3.2 below).

Restrictions in terms of susceptible strains are a significant limitation to the use this model since CII-permissive MHC is required. Still, CIA has proven to be very useful in drug development. For example, the importance of the IL-6 pathway was demonstrated in CIA using IL-$6^{-/-}$ mice [321]. TNF-α blockage and anti-IL-1 were also shown to be effective in CIA [322, 323]. Finally, blockage of B7 co-stimulatory molecules using CTLA4-Ig was also effective in CIA [324].

2.3.2 Collagen antibody-induced arthritis (CAIA)

CAIA is a mouse model in which arthritis is induced using a cocktail of CII-specific monoclonal antibodies followed by innate stimulation (e.g. LPS injection). This model relies on the understanding of CII epitopes recognized in CIA, and demonstrates directly the pathogenicity of anti-CII antibodies at high concentrations [325, 326].

Unlike CIA, this model is not MHC-restricted. In fact, there is a strong non-MHC genetic contribution to disease susceptibility. Both B cells and T cells are not essential. On the other hand, FcγRs are very important in modulating disease susceptibility [327]. In terms of cellular effectors, neutrophils depletion prevents disease development, whereas C5-deficient mice are partly protected, both demonstrating a crucial role of neutrophils [328]. Macrophages are also essential in CAIA [329]. A mice study using $C3^{-/-}$ and factor B $(FB)^{-/-}$ strains further revealed an implication of both the classical and alternative activation of the complement system [330]. Finally, both IL-1 and TNF-α were demonstrated to be implicated in the pathogenesis of CAIA using knockout mice, which is in accordance to RA [331]. However, $IL6^{-/-}$ mice had normal CAIA.

The CAIA model is mostly useful to study the effector phase of joints inflammation. It can be advantageous in drug screening due to its quick onset, and also its relative ease of use regarding susceptible strains (no MHC restriction) [332].

2.3.3 K/BxN serum transfer

K/BxN mice express the TCR transgene KRN that was designed to recognize the bovine ribonuclease peptide ($RNase_{42-56}$) on MHC I-A^k [333]. Unexpectedly, this TCR transgene recognizes a peptide from the ubiquitous glucose-6-phosphate isomerase (GPI) on the MHC II-A^{g7} derived from the NOD strain. This reactivity to GPI makes these mice susceptible to spontaneous arthritis. Although the model is T cells and B cells dependent, serum transfer from these mice is sufficient to induce arthritis in lymphocytes deficient recipients. Interestingly, immunization with GPI can also induce arthritis, a model implicating the IL-6/IL-17 pathway [334, 335].

Similarly to CAIA, TNF-α and IL-1 were found to contribute to joints inflammation upon antibodies transfer in knockout mice, whereas IL-6 did not [331]. Moreover, neutrophils depletion protected mice against K/BxN serum transfer, potentially by preventing the production of LTB_4 from neutrophils [336, 337]. Finally, FcγRs (in particular FcγRIII) and complement activation are important for the pathogenicity of sera [338].

2.3.4 Pristane induced arthritis (PIA)

An interesting and intriguing model of arthritis is the pristane-oil induced arthritis PIA in rats [339]. Both MHC and non-MHC genes influence this disease. The exact pathogenesis, including the disease specific antigens, remains to be fully understood. Nevertheless, the disease inducer (pristane) is a well-defined molecule, which after injection causes a chronic relapsing arthritis in susceptible strains with a prevalence of virtually 100% [340]. Studies using irradiation followed by repopulation revealed that $CD4^+$ T cells are needed for disease development, whereas B cells and $CD8^+$ T cells are not [341]. T cells activated *in vivo* can induce PIA in recipient rats upon transfer, confirming the central role of pathogenic T cells in this model [342].

2.3.5 The SKG model

The SKG model was originally described by Sakaguchi et al. in 2003 [343]. A spontaneous point mutation which occurred in a BALB/c colony made these so-called SKG mice susceptible to spontaneous chronic arthritis when kept in conventional animal facilities. This non-synonymous mutation in *ZAP70* (W163C) reduces TCR signaling and alters thymic selection, leading to leakage of arthritogenic $CD4^+$ T cells in the periphery of homozygote mutants [344]. Figure 9 illustrates the shift of T cells repertoire towards self-reactivity in SKG mice. This intrinsic defect in lymphoid progenitors was shown to still be pathogenic after transfer, even in mice of a different genetic background [343, 345]. Studies have revealed that the precise degree of reduction in TCR signaling is critical to arthritis susceptibility [344, 346]. In this thesis, we investigated the establishment of the SKG model in black strains with the MHC H2-A^q haplotype (Paper II).

2.3.5.1 Self-antigens in SKG mice

The root of autoimmunity in SKG mice is clearly demonstrated to be self-reactive $CD4^+$ T cells that escape central tolerance mechanisms. However, it remains unclear what are the key self-antigens triggering T cells activation. Early publications have identified several self-antigens targeted by antibodies, such as CII and heat shock protein-70 (HSP-70). Unlike with the K/BxN model, transferring sera does not confer arthritis, suggesting that these antigens might not necessarily be driving joints inflammation [343]. Due to the importance of CII reactivity in arthritis, we conducted a detailed investigation on CII as a potential self-antigen in SKG mice (Paper III).

In arthritic SKG mice, an increased frequency of T cells expressing TCR Vβ2 and TCR Vβ8.2 was observed, suggesting their implication in the disease development [347]. In an attempt to better comprehend this self-reactivity, two T cell clones were created from joint infiltrating T cells [348]. Both clones could lyse syngeneic synovial cells. However, one clone could also specifically lyse other MHC-matched cells, suggesting reactivity against a ubiquitous antigen. Interestingly, the two clones could induce independently arthritis and pneumonitis when transferred in nude recipients. More recently, Ito et al. identified self-reactivity against the ubiquitously expressed 60S ribosomal protein L23a (RPL23A) [349].

This finding is particularly interesting because self-reactivity against this protein is also found in T cells and sera of RA patients [349]. These results reinforce the notion that self-reactivity observed in SKG mice is relevant to human RA. However, identification of a ubiquitous antigen raises questions as to why joints are particularly affected in SKG mice.

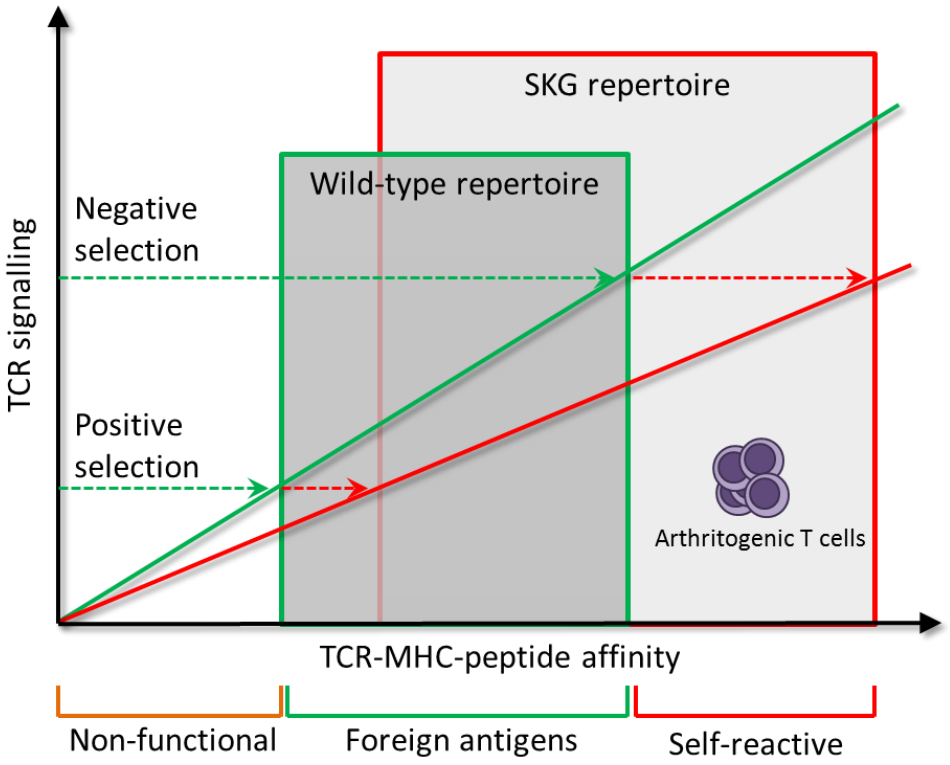

Figure 9 – Defective central tolerance in SKG mice due to deficient TCR signaling

Thresholds of intracellular TCR signaling strength determine the faith of thymocytes during T cells positive and negative selections. In wild-type animals, the selections will result in the removal of non-functional and self-reactive T cells. In SKG mice however, reduced TCR signaling alters the equivalent TCR affinity towards MHC-peptide complex needed for T cells to pass selections, leading to a general shift of repertoire towards self-reactivity.

2.3.5.2 A model sensitive to the environment

Spontaneous arthritis onset is not observed in SKG mice kept in specific pathogen free facilities (SPF), suggesting that arthritis in this model is due to a combination of genetic and environmental factors, something mimicking human RA [350]. Despite the presence of arthritogenic $CD4^+$ T cells in the periphery of SKG mice kept in SPF, activation of innate immunity with specific adjuvant is first needed to trigger chronic arthritis. Studies are now primarily conducted in SPF facilities with innate triggering because it allows a better control

over experimental factors such as microflora and time of onset, thus increasing reproducibility between experiments and different research centers.

Screening of adjuvants revealed that the β-glucan zymosan A and poly(I:C), but not LPS, ConA, pristane, pertussis toxin, cyclophosphamide, anti-CD40 and rat-IgG, could induce significant arthritis with an incidence approaching 100% [351]. It was later found that other extracts from *Candida albican*, such as mannan and curdlan, were also efficient [352-354]. Paper I focuses on innate immunity activation by mannan in non-SKG mice.

2.3.5.3 Sex hormones

Another environmental factor well known to influence human RA is sex hormones. Epidemiological studies clearly demonstrate a higher prevalence in women, which could be explained by the effects of estrogens, androgens and progesterone on inflammation [206, 355]. Early studies revealed a bias towards higher severity in female SKG mice, similarly to human RA, prompting some groups to further investigate sex hormones in SKG arthritis [343]. Sex does not only influence joints inflammation, but also periocular inflammation [356]. Testosterone is protective in SKG arthritis [357]. Pregnancy improves arthritis severity, similarly to remission observed in human [358, 359]. A different group also demonstrated that ovariectomy enhanced SKG arthritis, whereas 17β-estradiol treatment was slightly protective [360].

2.3.5.4 Effects of joint inflammation on bones

Several groups used the SKG model to study the effects of chronic inflammation on bones. Chronic inflammation in SKG mice led to reduced mechanical strength of bones, and disturbed collagen network organization, most likely due to increased bone turnover [361, 362]. Increased bone resorption was associated to increased number of osteoclasts on bone surfaces [363]. Further investigation identified the presence in the bone of osteoclast precursors derived from bone marrow myeloid population [364]. These osteoclast precursors have osteoclastic activity, and share features of both M1 and M2 macrophages. Surprisingly, they have suppressive effects on T cells, and can decrease the severity of arthritis in a SKG adoptive transfer model. Due to the effect of SKG arthritis on bones, the SKG model was also used to evaluate the impact of therapy on bones, and also the effect of therapy specifically against bone resorption [365, 366].

2.3.5.5 Other sites of inflammation

Although joints are the primary sites of inflammation, various groups have also described other forms of autoimmunity accompanying arthritis in SKG mice. This is not surprising considering that the mechanism leading to autoimmunity is defective central tolerance of T cells. Hence unlike in CIA for example, it is not specifically targeting joints. However what is surprising is that the adjuvant used, and potentially the microflora, alters the organs affected by autoimmunity. For example, it was found that curdlan, but not mannan, also induces uveitis and ileitis resembling Crohn's disease which seems to be caused by changes in the gut

microflora [354, 367, 368]. A different group recently described both periocular and lung inflammations with both curdlan and zymosan A, but not uveitis [356]. Lung inflammation itself had already been described by various other groups [348, 369, 370]. Finally, skin infiltrates were observed in arthritic SKG mice kept in conventional animal facility, however mannan itself was also found to cause dermatitis even in non-SKG mice, potentially confounding any further investigations [343, 371]. These disparate autoimmune profiles are not necessarily contradictory, but only reinforce the sensitivity of this model to environmental factors such as microflora. Nevertheless, joints seem to be particularly susceptible to inflammation since no group reported an absence of arthritis.

2.3.5.6 Cytokines network in SKG arthritis

There is major interest in understanding the cytokines network associated with arthritis in SKG mice, partly because of the therapeutic importance of cytokines blockage in human RA, such as TNF-α blockers [372, 373]. It was established early that IL-6, IL-1 and TNF-α were overexpressed in arthritic synovial tissue, and that their absence in knockout mice greatly reduced, or even abolished, arthritis onset in SKG mice, whereas the lack of IL-10 leads to increased disease severity [374]. The increase in IL-6 levels was also observed in zymosan A treated mice [375]. However, it was somewhat surprising that neither IFN-γ nor IL-4 influenced arthritis in SKG mice, which did not fit with the reigning Th1/Th2 paradigm at that time [374].

Understanding the role of IL-17 in arthritic SKG mice was a major breakthrough in understanding the underlying cytokines network operating [376]. Similarly to human RA, the major source of IL-17 in synovium is αβT cells in the SKG model, and not γδT cells like it is in CIA [377]. In fact, γδT cells in SKG mice are deficient in terms of IL-17 production due to the mutation in *ZAP70* [378]. These Th17 cells express CCR6 and are recruited in joints by CCL20 produced by synoviocytes [379].

Subsequent studies revealed that exposure to zymosan A leads to the secretion of GM-CSF, which in turn activates macrophages production of IL-1β and IL-6 [369]. The resulting cytokines microenvironment favors Th17 differentiation and further production of GM-CSF and IL-17. The activation of macrophages and subsequent Th17 differentiation is dependent upon complement activation of C5a [352]. As mentioned by the authors, C5a, IL-17 and GM-CSF are all potent enhancers of granulopoiesis and will cause the recruitment and accumulation of neutrophils in inflamed tissues. The lack of ROS burst due to defective NOX2 complex in SKG.Ncf1$^{m1j/m1j}$ mice was also shown to increase accumulation of neutrophils in inflamed joints (Paper IV).

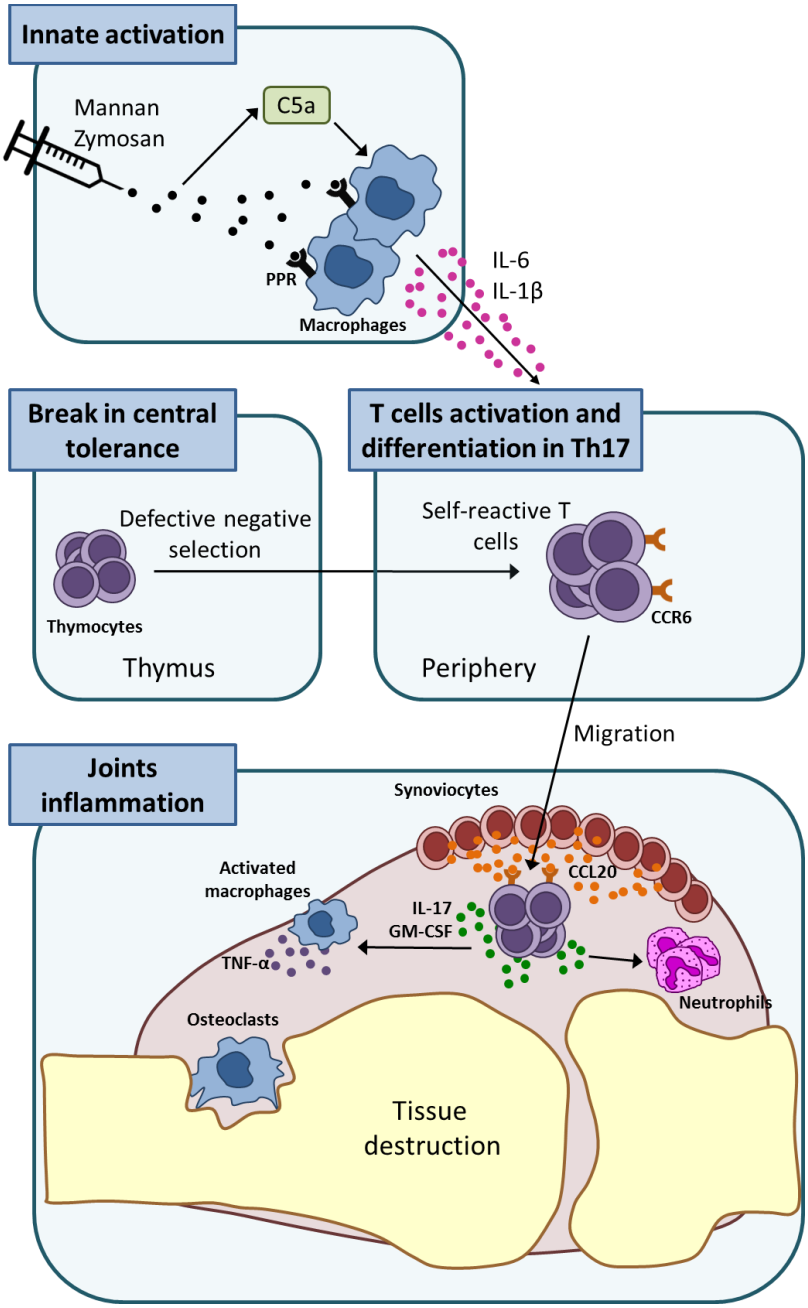

Figure 10 – Key mediators of arthritis in SKG mice after zymosan A or mannan injection

Defective central tolerance in SKG mice causes the leakage of self-reactive T cells in the periphery. Activation and recruitment of these T cells are however necessary to induce joints inflammation. A single injection of zymosan A or mannan leads to macrophages activation via complement C5a and various PRRs. Production of IL-1β and IL-6 lead to T cells activation and differentiation into Th17. Local secretion in the joints by synoviocytes of the chemokine CCL20 recruits Th17 expressing CCR6 in the joints where they secrete IL-17 and GM-CSF. Both cytokines help to the recruitment and activation of neutrophils and monocytes while also inducing granulopoiesis. Secreted enzymes from these cells, and from osteoclasts, slowly erode joints.

Neutralization of either GM-CSF or IL-6, but not IL-17, greatly reduced lungs inflammation [369]. Joints inflammation itself was only slightly reduced by antibody neutralization of IL-17, however a different group later demonstrated that in SKG.IL-17$^{-/-}$, there is a clear reduction in joints inflammation and ileitis [368]. The same group also highlighted the importance of IL-23, also supporting the implication of Th17. This distinction between depletion with antibodies and knockout technology could be explained by a potentially incomplete depletion of IL-17 locally in inflamed tissues, supporting a local role of this cytokine in SKG arthritis.

Altogether, the cytokines network identified in SKG mice is relevant to human RA, as demonstrated by the similarities between these cytokines and the pathways targeted by biologic drugs in RA (e.g. TNF-α, IL-6, IL-1). In fact, except for rituximab which targets B cells, all biologic DMARDs approved in RA target pathways implicated in SKG arthritis [380]. Figure 10 proposes a cytokines network based on various publications.

2.3.5.7 SKG mice in drug discovery

Animal models are usually used for two distinctive, yet critical, reasons. The first is to dissect mechanisms of arthritis and get valuable information on the biological problem. The second is to investigate novel therapeutic interventions. Animal experiments are ideal to evaluate novel interventions in highly controlled conditions compared to clinical studies, and allow evaluation of therapeutic approaches using genetic modifications before having to develop a molecule. Highly controlled conditions reduce variability, and thus reduce the number of subjects needed to detect statistically significant effects. Moreover, knockout mice represent absolute suppression of a particular pathway; hence they give an indication of the maximal efficacy possible to reach because due to pharmacological limitations, a drug is likely to not fully inhibit a certain pathway.

Altogether, animal experimentations are an essential step between *in vitro* experiments and clinical trials as it allows a complex *in vivo* proof-of-mechanism while minimizing the risk for human subjects. However, a critical question to address is how good an animal model is at predicting safety and efficacy in human due to interspecies discrepancies, and the inherent shortcomings of modeling a disease.

The relevance of an animal model is often measured by its ability to predict efficacy of treatments in human. There exists however a bias in this analysis, especially with older models such as CIA, because only drugs effective in these models will have been further investigated in the first place. This bias is absent in newer models, such as the SKG mice, since most drugs were discovered before the model. However, being newer, these models are not as well understood which could also be problematic. A prudent approach to circumvent these issues is to use a combination of independent models where different immunological pathways are involved. For instance, using both CAIA and K/BxN sera transfer, or CIA and CAIA, provides little additional information considering their similarities. On the other hand,

the addition of the SKG model to CIA helps at eliminating potential bias due to immunization (e.g. directing the adaptive immune response to a specific antigen), while still addressing the production of pathogenic self-reactive antibodies.

Due to the high incidence and the rapid onset of arthritis in SKG mice, it presents many practical advantages to study novel therapies to treat RA. The mechanisms leading to arthritis and several features of arthritis in SKG mice are very relevant to human RA. Limited knowledge and expertise in the SKG model, the need for homozygote mutants and restrictions in available strains might have slowed the implantation of the SKG model in arthritis research. Nevertheless, several studies have already used this model, particularly in the past 5 years, to evaluate either novel or existing treatments [380-390]. In fact, tofacitinib – the first Janus kinase (JAK) inhibitor approved for RA and the most recently FDA approved innovative drug (February 2016) – was also tested in SKG mice prior to its launch [380]. It is now reasonable to think that this increased use of the SKG model will continue in the next few years. It will be interesting to follow the outcome of these novel therapeutic approaches to see the predictive value of the SKG model. A systemic evaluation of existing drugs in SKG mice would also provide valuable information regarding its predictive value.

3 PRESENT STUDY

This thesis includes 4 manuscripts, all of which dealing with autoimmunity in mice. The focus is on chronic arthritis in SKG mice induced by mannan. Paper I describes the innate immunological events following mannan injection in non-SKG mice. Paper II, III and IV then use this innate stimulation as a trigger to chronic Th17-driven arthritis in SKG mice. Paper II describes the similar autoimmune profile observed in SKG mice on the B6.Q and B10.Q genetic backgrounds. Paper III studies the role of CII as a potential self-antigen in SKG arthritis. And finally, paper IV investigates the regulatory role of ROS in autoimmunity.

3.1 PAPER I: MANNAN INDUCES ROS-REGULATED, IL-17A–DEPENDENT PSORIASIS ARTHRITIS-LIKE DISEASE IN MICE

Mannan is a polysaccharide extracted from the wall membrane of yeast. It is believed to activate innate immunity through various PRR, including dectin-2 receptors [29]. This publication describes how mice exposed to a high dose of mannan i.p. experience transient joints and skin inflammations. This finding is interesting considering that a combination of joints and skin inflammation mimics psoriasis and psoriatic arthritis, and thus represents a new model of this disease.

This publication highlights the importance of ROS as regulators of inflammation since skin and joints inflammation in $Ncf1^{m1j/m1j}$ mice lacking NOX2-dependent ROS burst is greatly enhanced. Neither B cells nor αβT cells were essential; however macrophages played a significant role. Further investigation revealed that IL-17A and neutrophils were critical to both skin and joints inflammations. Considering the absolute protection in $RAG^{-/-}$ (data not shown), and that γδT cells, from both the peritoneal cavity and the skin, were found to produce high amounts of IL-17A upon *in vivo* stimulation, we postulate that these cells were likely implicated in the pathogenesis of this novel animal model of psoriasis. However, direct causality, for instance by γδT cells depletion or knockout mice, was not demonstrated.

Understanding the inflammatory cascade following mannan injection is critical since it leads to the activation of pathogenic SKG T cells in SKG mutant mice, and the subsequent chronic arthritis. In non-SKG mice, such as in this publication, the inflammation self-resorbs due to the lack of pathogenic SKG T cells. It is worth noting however that in SKG mice, γδT cells are severely deficient in terms of IL-17A production and are therefore unlikely to be a significant acute source of IL-17A upon mannan stimulation (data in supplementary figures of paper II). Hence, the acute source of IL-17A remains unknown in SKG mice.

3.2 PAPER II: THE SKG MUTATION IN *ZAP-70* ALSO CONFERS ARTHRITIS SUSCEPTIBILITY IN C57 BLACK MOUSE STRAINS

The SKG model was originally discovered in a BALB/c colony [343]. Similarly to the *PTPN22* mutation, the *ZAP70* mutation leads to reduction in TCR signaling and is unspecific in terms of autoimmunity. In SKG.BALB/c mice, it led to joints inflammation; however it is unclear why joints in particular are affected, similarly to human RA. The effect in terms of

autoimmune profile of this point mutation in a different genetic background or a different MHC haplotype was thus unknown. Before this study, no other strains had been used to study SKG arthritis. Having the SKG model available in black strains, in particular the B6 strain, has significant importance considering the wide use of this background for genetically modified strains. Moreover, most arthritis research is conducted in mice bearing the MHC H2-A^q haplotype due to their susceptibility to CIA.

Therefore, in this publication, we describe the backcrossing over more than 10 generations of the SKG mutation on the B6.Q and B10.Q genetic backgrounds. Mice retained impaired TCR signaling as demonstrated by reduced calcium influx and reduced proliferation following anti-CD3/anti-CD28 stimulation. Similarly to SKG.BALB/c, this reduced signaling had a profound effect on thymic selection, reducing output from both DN3 to DN4, and more significantly from DP to SP. Arthritogenic T cells hence escaped thymic selection, leading to susceptibility to chronic T cell driven arthritis upon activation of the innate immune system with mannan. Imputability of T cells was demonstrated using adoptive transfer. The SKG mutation led to aberrant T cells activation with a strong bias towards Th17, and production of self-reactive antibody against CII.

Results in this manuscript should hopefully help to spread the use of the SKG model by other research groups. These results were also the foundation of the two other manuscripts in this thesis since it allowed complex crossing with the large collection of transgenic strains on the B10.Q genetic background used in our research group.

3.3 PAPER III: T CELL SPECIFICITY AGAINST GALACTOSYLATED CII ENHANCES COLLAGEN INDUCED ARTHRITIS IN SKG MICE BUT DOES NOT AFFECT ADJUVANT INDUCED ARTHRITIS

As demonstrated by research on ACPAs in RA, biomarkers research in arthritis is essential to understand the disease. Moreover, understanding the role of specific self-antigens is essential in the development of targeted therapeutic interventions such as vaccination. The role of CII as a potential self-antigen, and of anti-CII antibodies, are thus of significant importance. In human, anti-CII antibodies are found in a subset of patients with rapidly progressive RA [391, 392]. Moreover, in animal models of arthritis, high reactivity against CII is known to be pathogenic, as demonstrated by CIA and CAIA [309, 325, 326]. However, the contribution of low self-reactivity against CII in the development of arthritis still remains unknown.

The SKG model represents a unique opportunity to address this question considering that these mice develop spontaneously anti-CII antibodies after mannan injection, despite the lack of immunization with CII. To fully address the role of CII reactivity in this model, SKG mice were first crossed with CII-permissive MHC H2-A^q. Then, they were crossed with CII-specific βTCR (Vβ12) and αβTCR (HCQ.3) transgenic strains to enhance CII reactivity. Finally, T cells repertoire was fixed to react solely against CII by fixing the repertoire to HCQ.3 using $RAG^{-/-}$.

Increased T cells reactivity against CII and expression of MHC H2-A^q made SKG mice susceptible to CIA. An expansion of CII-specific T cells is observed after CII immunization in SKG.Vβ12, whereas a large pool of CII-specific T cells is already presents even in naïve SKG.HCQ.3 mice. Despite these observations, both SKG.Vβ12 and SKG.HCQ.3 are equally susceptible to arthritis following mannan injection compared to SKG mice, suggesting that T cells reactivity against CII has no effect in this model. Furthermore, fixing T cells repertoire to CII reactivity abolishes mannan induced arthritis, supporting that reactivity against other antigens is what drives chronic arthritis in SKG mice.

Altogether, this publication highlights the complementarity of CIA and SKG arthritis. Indeed, CIA-promoting transgenes such as Vβ12 and HCQ.3 had no effect in SKG mice. Such distinction is interesting since it demonstrates that findings from CIA could greatly benefit from an independent evaluation in SKG mice. Finally, since increasing CII reactivity did not alter arthritis susceptibility in SKG mice following mannan injection, it suggests that the observed anti-CII antibodies are not a crucial part of this disease model, raising questions regarding their implication in human RA.

3.4 PAPER IV: ROS REGULATE INNATE BUT NOT ADAPTIVE INFLAMMATION IN ZAP70 MUTATED SKG ARTHRITIC MICE

The antimicrobial role of ROS produced by neutrophils and macrophages is essential for host defense [393]. In human, defects in NOX2 lead to a rare immunodeficiency named chronic granulomatous disease (CGD) characterized by apparition of granuloma in various organs due to the incapacity of phagocytes to kill bacteria [394]. In chronic inflammation however, such as in arthritis, the role of ROS is traditionally believed to be deleterious because it is thought that prolonged tissue exposure to ROS can cause collateral tissue damage.

This pathogenic role of ROS in arthritis was seriously challenged when genetic studies revealed that an arthritis promoting polymorphism in rats was a mutation within *Ncf1* leading to reduction in ROS burst by phagocytes [395]. This disease enhancement by deficient ROS burst was confirmed in $Ncf1^{m1j/m1j}$ and $GP91^{-/-}$ mice, in which susceptibility to CIA was greatly enhanced despite the abolition of NOX2 activity [396, 397]. Interestingly, CGD patients were later found to have an increased risk of autoimmune diseases compared to the general population [398].

Despite this clear demonstration that a lack of ROS from phagocytes promotes arthritis, the mechanism leading to down-regulation of the immune response remained poorly understood. A previous study suggested that reduction of thiols on the surface of T cells in ROS-deficient rodents increase activation and arthritogenicity of T cells [399]. Furthermore, ROS-deficiency in $Ncf1^{m1j/m1j}$ mice led to break in T cells tolerance towards endogenous CII, however it was unclear if this was a central or peripheral effect [400]. Other studies suggested that ROS would exert their effect through innate immunity. For instance, activation of DC with *M. tuberculosis* leads to higher production of IL-6 and TFG-β, and a subsequent bias towards Th17 and increased CIA susceptibility in ROS deficient mice [401]. Finally,

macrophage-derived ROS were found to be protective in CIA, and also in B cell and T cell independent mannan-induced psoriatic arthritis [371, 402]. Altogether, these results demonstrate the need to further investigate the immunoregulation mechanisms of ROS.

The uniqueness of the SKG model gave us the perfect setting to revisit several hypotheses generated with previous arthritis models. *In vivo* imaging revealed a high burst of ROS production in arthritic joints of SKG mice, followed by steady, yet abnormally elevated, ROS levels in inflamed joints. To characterize the effect of ROS in SKG arthritis, SKG.B10.Q mice were crossed with Ncf1$^{m1j/m1j}$ mice to generate ROS deficient SKG.Ncf1 $^{m1j/m1j}$ mice. As observed in CIA, ROS deficiency greatly enhances arthritis susceptibility of SKG mice. This increase in arthritis severity is associated to elevated concentrations of anti-CII antibodies in the blood; however transfer experiments demonstrated that the sera were not pathogenic. Regarding T cells, no difference were observed regarding thymic selection or T cells activity in the periphery in absence of ROS. Adoptive transfer of peripheral T cells revealed a crucial role of peripheral ROS. Using transgenic mice expressing functional Ncf1 under the hCD68 promoter, it was found that macrophage-derived ROS can regulate arthritis. Altogether, this publication highlights the role of macrophage-derived ROS in regulating T cell driven autoimmunity independently of adaptive immune response, which has therapeutic implications.

3.5 PERSPECTIVES

Paper I introduces a new model of psoriatic arthritis and identifies a potential disease mechanism. Although the role of IL-17 was clearly demonstrated, and despite that γδT cells seem to be a potent source of it after mannan stimulation *in vivo*, it remains unproven that they are key contributors in this disease model. The absolute abolition of disease susceptibility in RAG$^{-/-}$ supports this idea. However, SKG mice are equally susceptible to mannan induced psoriasis-like lesions, yet their γδT cells are severely deficient in terms of IL-17 production. This suggests that at least in SKG mice, another major source of acute IL-17 production exists. The use of knockout strains and depleting antibodies could provide the answer to this question.

Considering that it is a new model, it would be interesting to study the response to various treatments used in psoriasis and psoriatic arthritis. Indeed, this model presents many practical advantages in drug screening (e.g. fast onset, high incidence), however the relevance to human is still unknown. On the other hand, the very short duration of this model might be problematic. Although the lack of a role for αβT cells in this model raises questions regarding the relevance of this model, downstream and effector mechanisms (e.g. IL-17 and neutrophils) might still mimic the human pathology. Demonstrating a good predictive value of this model in hence the required next step to make the model attractive to researchers.

To our knowledge, **paper II** is the first report that the SKG model can be used on a different genetic background than BALB/c, including also a different MHC haplotype. The method used was insufficient to exclude an effect of MHC haplotypes on disease severity. Systemic

screening of various MHC, along with backcrossing of the SKG mutation to other mice strains could represent an interesting tool to detect arthritis promoting genes. A similar method with other animal models was used to identify several risk loci (e.g. polymorphism in *Ncf1*). In the meanwhile, due to its uniqueness, the SKG.B10.Q mice model can be used to further investigate previously identified disease promoting polymorphisms. This thesis presents two projects which focused on previously identified factors enhancing CIA: the CII reactivity of T cells, and the lack of NOX2-derived ROS burst.

Considering the results of **paper III**, it does not seem appropriate to further study the role of CII in SKG mice. Instead, future investigations should focus on identifying other antigens driving chronic arthritis in SKG mice. The fact that the model could be transferred to a new MHC suggests either that several redundant antigens are implicated, or that the self-antigen binds both MHC molecules. Further crosses to new MHC molecules could help to investigate this question and thus help to identify pathogenic self-antigens. Regarding the role of CII reactivity in human RA, although this study raises questions, it is insufficient by itself to draw any firm conclusions.

Finally, regarding the immunoregulation of arthritis by ROS described in **paper IV**, the ultimate goal of this large endeavor is the development of a drug using this anti-inflammatory function of macrophage-derived ROS to treat RA. However, several key questions remain unanswered. An urgent issue is to exclude the possibility that ROS either directly or indirectly induce pain. Another important issue is to demonstrate that the observed suppressive effect of ROS can be further increased with supra-physiologic ROS production. In fact, we were only able to demonstrate that the absolute lack of ROS burst from phagocytes enhances arthritis, and that restoring ROS production in macrophages restores normal arthritis susceptibility.

Moreover, it would be interesting to identify the precise oxidative molecules suppressing inflammation. This would help in the development of therapeutic approaches to alter the redox balance. Instead of NOX-2 inducers, directing this balance towards specific oxidative molecules by inhibiting key conversation enzymes might be more appropriate. Finally, although macrophage-derived ROS from NOX2 were protective in arthritis, it does not exclude the possibility that other cellular sources of ROS (e.g. neutrophils) or that ROS from other NOX complexes could have a similar role.

The mechanism of ROS-induced immunosuppression is still not fully understood, but two avenues are particularly relevant to investigate at this point. It appears that the underlying mechanism is fairly broad since many disease models are affected by ROS deficiency: from innate model like mannan induced psoriasis, to B cell and T cell dependent model such as CIA. It could also be that ROS operates by various mechanisms in different models, which would greatly complicate further investigations.

The first mechanism to investigate is the local survival of cells, in particular effector cells such as neutrophils, in inflamed tissues. Excess of ROS is known to induce apoptosis [403,

404]. This could represent a physiological negative feedback mechanism to limit ongoing tissue inflammation, and aberrant ROS deficiency could disrupt it.

The other mechanism to investigate is the polarization of macrophages. Local ROS levels could influence differentiation of macrophages from M1 to M2 phenotype. Again, this mechanism would be logical from a physiological perspective since a shift to tissue repairing M2 phenotype following tissue inflammation would help the organism recover from infections. ROS were shown to be critical in alternative differentiation of macrophages and generation of tumor-associated macrophages, which strongly support a similar role in autoimmunity [405]. Hence, the various mice strains used in the lab to study the role of ROS in autoimmunity could also be very useful in cancer research.

3.6 CONCLUDING REMARK

In conclusion, the SKG model has received growing interest from the arthritis research community ever since its original publication in 2003. Features relevant to human RA, along with a unique pathogenesis, make this model an interesting tool for researchers to study biological questions related to arthritis and autoimmunity. The increasing use of the SKG model in drug discovery in the past few years should reveal how relevant the model is to human RA. Hopefully, the work presented in this thesis should facilitate the use of this model by various research groups, as well as contribute to the overall understanding of autoimmunity.

4 ACKNOWLEDGEMENTS

It feels as if it was only yesterday that I left Switzerland for Sweden. Still, in these 4 years, a lot of things have happened, some good and some bad. I met great people, have worked hard and learned a lot about research and myself. Of course, like anyone else, I faced numerous challenges over these years, but thanks to the support I received, I am now here today, finishing my PhD and about to engage in a new adventure. I would like to take the time to acknowledge everyone who has made this possible.

First, thank you **Kajsa** for giving this great opportunity. I have grown as scientist over the past 4 years, and this is thanks to you. You trusted me and gave me a lot of scientific freedom while still always taking the time to discuss with me about my projects. I hope the experience was as positive for you as it was for me. My PhD wouldn't have been the same without the advices and knowledge of **Rikard**, which also created a unique environment for research (MIR), providing all the tools, resources and expertise needed for research of the highest quality. Also thank you **Liselotte** for your support and advices when I needed it. Although not being directly implicated in my projects, thank you to both **Nan** and **Johan** for your expertise and your various management responsibilities in the group.

I would also want to acknowledge all my previous mentors which prepared me for a PhD at Karolinska Institutet. Thank you **Patrice** for introducing me to immunology and arthritis. **Roxane** for a great master opportunity and your constant supports, even nowadays. Also, thank you **Jean-Christophe** for this great opportunity at ETH and your understanding after I decided to leave drug delivery. Thank you also to **Marc** and **Bastien** for your help and support in organic chemistry.

Thanks also to all my **ex-colleagues at ETH** for all the good times; I hope we meet again soon. It was great to meet again **Nancy**, and you are most definitely welcome wherever I end up. **Elisabeth** for all these nice conversation and being so good at keeping in touch. **Stefan**, you were an awesome flatmate and I really miss going out with you and **Julia**. Finally, thank you **Fabienne** for the great times we had together, and for helping me make this big decision, find the courage to do it and support me in my decision.

To **all my colleagues at MIR**, past and present, which made those years at MIR a unique experience – thank you. **Erik** of course, always in the mood for interesting discussions, and who has always been very supportive from the start to the end. We worked a lot together, but most importantly, we had great evenings. I'm really happy for you and **Nastya**. It's hard to think of those evenings without remembering the good times with both **Florian** and **Clara**.

Mike and **Gonzalo** for being so original, unique and fun. Thank you **Daniëlle** for all the good times and running toughest Copenhagen with me.

Thanks **Katrin** for showing me around when I first arrived in the lab, and for taking care of my mice while I was away. **Min** for your enthusiasm, your great science and your honesty. Thanks to you and Lena for our small collaboration about neutrophils. **Liesu** for always smiling, even after long days down at the animal house. Thank you **Amit** for always being in a mood for a joke.

A general thank you to the Chinese community in the lab for sharing with us so many dumplings (**Bibo, Dongmei, Naru, Changrong, Lilu, Bingze, Wenhua**)! Big thanks to **Jianghong** for all your advices and our work together at setting up *in vivo* imaging and studying L-NAME. **Cecilia** for the collaborations on CAIA mannan. **Anthony** for your advices on accommodations in Stockholm. **Outi** for the TN3 mice. **Vilma** for the help with nitrogen tanks. **Ulrika** for tolerating my eating habits in the office.

Thank you **Angel** for all the administrative help over the years. All this animal work was possible thanks to great animal care by **Carlos the Boss**, **Kristina** and all the other technicians over the years. **Biborka** for your role with the veterinarian and ethical issues: you've always been helpful and comprehensive. **Emma** for the great histology. **Susanne** for making the lab a little more functional. I'd like to acknowledge past members too – **Susann, Bruno, Sabrina, Jonathan, Ingrid, Martina and Ia** - for all the wise advices about both research and career.

In 4 years in Sweden (and 5 in Europe), I have met a lot of great people. It is impossible to name everyone, I'm really sorry, but of course all you fantastic people from both national schools in **DDD** and **NCRSCID**. Also, I was fortunate enough to meet wonderful people at the **FEBS 2013** immunology course in Croatia, **SIICA 2015** in Catanzaro, and more recently **REDOX 2016** in Nebraska. And also at national conferences like **Basic to Clinic** and **KiiM retreat**. Thank you also to **Anna** and the whole team at **Scandinavian Development Service** for this great opportunity to learn drug development from the best.

Although I am excited to start soon with new challenges, leaving Stockholm and **my friends** is quite sad and I will miss you all. Thank you **Joanna** and **Milind** for all the great evenings and support over the years. The week in Greece was amazing! **Emma** for all the nice chat about basically anything – even science sometimes! Thanks **Maria** for all those nice fika, and of course for basically taking me off the street. **Christina** for all the great fun and being such a good friend. **Soaz** for the great evenings and delicious crepes. **Francesca** for all the biscuits.

Sara for the best cover idea ever, and all the great times with the "crew" from Science to Business (**Kata, Alex, Öykü, Leo, Sabrina**). **Cynthia** and **Anna** for ice-skating and fun in general.

A general thank you to all my friends spread around Europe which took time to show me wonderful places and discover different cultures. In particular **Pauline** for interesting conversations about medicine and showing me Paris. **Lilia** for showing me Oslo and being so outspoken. And of course, thank you **Marta** for all the great times, and being so enthusiastic to show me the real Barcelona – I have unforgettable memories. I hope our paths cross again soon, but you'll always be welcome wherever I go.

Merci à tous mes amis au Québec qui prennent le temps quand je suis de retour. **Sam**, **Nicolas** et **Alex,** merci de toujours prendre le temps pour une bière (ou 2) quand je suis à Montréal. **Alex** et **Anne-Marie** également, ça fait toujours plaisir de vous voir! **Maryves**, **Annie** et **En**, dommage que je sois si loin, mais pharmacie ensemble c'était franchement cool. Merci **Lorène** pour toutes ces bonnes soirées à Québec : dommage que l'Arabie Saoudite soit si loin mais on va s'arranger! Et **Minh-Tan** avec tes nombreuses passions et discussions intéressantes.

Un gros merci à toute **ma famille** qui m'a toujours supporté malgré la distance. Les réunions de famille au lac ou pour le jour de l'an me manquent beaucoup, et j'apprécie qu'on prenne le temps de se voir quand je suis de passage. Merci **Papa** pour ces beaux voyages en nature ensemble au Québec – mes passions pour la photographie et le plein air viennent de cela. **Valérie**, merci ma petite grande sœur qui m'inspire à voir la vie d'un point de vue original, et qui prend le temps de m'écouter parler de ma recherche et réexplique fièrement le tout à ses amis. Merci **Maman** et **Cristian**, en particulier pour la bonne bouffe et les bonnes soirées qui sont une source d'énergie inestimable quand je suis de retour. Et **Lucia** bien sûr, merci de tout cœur de m'avoir épaulé dans les moments les plus durs et pour tous tes conseils. J'espère que tu sais à quel point, malgré la distance, tu as contribué à mon succès.

Finally, all of this wouldn't have been possible without **Margherita**. We've met at the perfect time in my life, and since then, you have always been there to support me, even in the toughest times. Your family has been very nice with me and I would like to thank them all too (**Paolo**, **Nicoletta**, **Francesco**). Your support, both with the science and everything else, has made a huge difference. Thank you.

5 REFERENCES

1. Murphy, K.P., et al., *Janeway's Immunobiology*. 2008: Garland Science.
2. Abbas, A.K. and A.H. Lichtman, *Basic immunology : functions and disorders of the immune system*. 3rd ed. 2009, Philadelphia, PA: Saunders/Elsevier. viii, 312 p.
3. Barnes, P.J., *Immunology of asthma and chronic obstructive pulmonary disease.* Nat Rev Immunol, 2008. **8**(3): p. 183-92.
4. Finn, O.J., *Cancer immunology.* N Engl J Med, 2008. **358**(25): p. 2704-15.
5. Park, J.E. and A. Barbul, *Understanding the role of immune regulation in wound healing.* Am J Surg, 2004. **187**(5A): p. 11S-16S.
6. Ross, R., *Atherosclerosis--an inflammatory disease.* N Engl J Med, 1999. **340**(2): p. 115-26.
7. Hansson, G.K. and A. Hermansson, *The immune system in atherosclerosis.* Nat Immunol, 2011. **12**(3): p. 204-12.
8. Rosenberg, S.A., J.C. Yang, and N.P. Restifo, *Cancer immunotherapy: moving beyond current vaccines.* Nat Med, 2004. **10**(9): p. 909-15.
9. Gatti, R.A. and R.A. Good, *Occurrence of malignancy in immunodeficiency diseases. A literature review.* Cancer, 1971. **28**(1): p. 89-98.
10. Toruner, M., et al., *Risk factors for opportunistic infections in patients with inflammatory bowel disease.* Gastroenterology, 2008. **134**(4): p. 929-36.
11. Kaplan, J.E., et al., *Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.* MMWR Recomm Rep, 2009. **58**(RR-4): p. 1-207; quiz CE1-4.
12. Anderson, J.M. and C.M. Van Itallie, *Physiology and Function of the Tight Junction.* Cold Spring Harbor Perspectives in Biology, 2009. **1**(2).
13. Nizet, V., et al., *Innate antimicrobial peptide protects the skin from invasive bacterial infection.* Nature, 2001. **414**(6862): p. 454-457.
14. Artis, D., *Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut.* Nature Reviews Immunology, 2008. **8**(6): p. 411-420.
15. Backhed, F., et al., *Host-bacterial mutualism in the human intestine.* Science, 2005. **307**(5717): p. 1915-1920.
16. Ouwehand, A., E. Isolauri, and S. Salminen, *The role of the intestinal microflora for the development of the immune system in early childhood.* Eur J Nutr, 2002. **41 Suppl 1**: p. I32-7.
17. Kelly, D., S. Conway, and R. Aminov, *Commensal gut bacteria: mechanisms of immune modulation.* Trends Immunol, 2005. **26**(6): p. 326-33.
18. Round, J.L. and S.K. Mazmanian, *The gut microbiota shapes intestinal immune responses during health and disease.* Nature Reviews Immunology, 2009. **9**(5): p. 313-323.
19. Akira, S., S. Uematsu, and O. Takeuchi, *Pathogen recognition and innate immunity.* Cell, 2006. **124**(4): p. 783-801.
20. Lanier, L.L., *NK cell recognition.* Annual Review of Immunology, 2005. **23**: p. 225-274.
21. Bauer, S., et al., *Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA.* Science, 1999. **285**(5428): p. 727-729.
22. Lu, Y.C., W.C. Yeh, and P.S. Ohashi, *LPS/TLR4 signal transduction pathway.* Cytokine, 2008. **42**(2): p. 145-51.
23. Matzinger, P., *Tolerance, danger, and the extended family.* Annu Rev Immunol, 1994. **12**: p. 991-1045.
24. Bianchi, M.E., *DAMPs, PAMPs and alarmins: all we need to know about danger.* Journal of Leukocyte Biology, 2007. **81**(1): p. 1-5.
25. Scaffidi, P., T. Misteli, and M.E. Bianchi, *Release of chromatin protein HMGB1 by necrotic cells triggers inflammation.* Nature, 2002. **418**(6894): p. 191-5.
26. Harris, H.E., U. Andersson, and D.S. Pisetsky, *HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease.* Nat Rev Rheumatol, 2012. **8**(4): p. 195-202.
27. Netea, M.G., et al., *An integrated model of the recognition of Candida albicans by the innate immune system.* Nat Rev Microbiol, 2008. **6**(1): p. 67-78.
28. Cambi, A., et al., *The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells.* Eur J Immunol, 2003. **33**(2): p. 532-8.

29. Saijo, S., et al., *Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans.* Immunity, 2010. **32**(5): p. 681-91.
30. Tada, H., et al., *Saccharomyces cerevisiae- and Candida albicans-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner.* Microbiol Immunol, 2002. **46**(7): p. 503-12.
31. Netea, M.G., et al., *Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors.* J Clin Invest, 2006. **116**(6): p. 1642-50.
32. Cambi, A., et al., *Dendritic cell interaction with Candida albicans critically depends on N-linked mannan.* J Biol Chem, 2008. **283**(29): p. 20590-9.
33. van de Veerdonk, F.L., et al., *The macrophage mannose receptor induces IL-17 in response to Candida albicans.* Cell Host Microbe, 2009. **5**(4): p. 329-40.
34. Sheng, K.C., et al., *Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells.* Immunology, 2006. **118**(3): p. 372-83.
35. Medzhitov, R., *Toll-like receptors and innate immunity.* Nat Rev Immunol, 2001. **1**(2): p. 135-45.
36. Takeuchi, O. and S. Akira, *Pattern recognition receptors and inflammation.* Cell, 2010. **140**(6): p. 805-20.
37. Randolph, G.J., et al., *Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo.* Immunity, 1999. **11**(6): p. 753-761.
38. Randolph, G.J., V. Angeli, and M.A. Swartz, *Dendritic-cell trafficking to lymph nodes through lymphatic vessels.* Nature Reviews Immunology, 2005. **5**(8): p. 617-628.
39. Banchereau, J. and R.M. Steinman, *Dendritic cells and the control of immunity.* Nature, 1998. **392**(6673): p. 245-252.
40. Murray, P.J. and T.A. Wynn, *Protective and pathogenic functions of macrophage subsets.* Nat Rev Immunol, 2011. **11**(11): p. 723-37.
41. Gordon, S., *Alternative activation of macrophages.* Nat Rev Immunol, 2003. **3**(1): p. 23-35.
42. Mantovani, A., et al., *Macrophage plasticity and polarization in tissue repair and remodelling.* J Pathol, 2013. **229**(2): p. 176-85.
43. Mosser, D.M. and J.P. Edwards, *Exploring the full spectrum of macrophage activation.* Nat Rev Immunol, 2008. **8**(12): p. 958-69.
44. Mantovani, A., et al., *The chemokine system in diverse forms of macrophage activation and polarization.* Trends Immunol, 2004. **25**(12): p. 677-86.
45. Sica, A. and A. Mantovani, *Macrophage plasticity and polarization: in vivo veritas.* J Clin Invest, 2012. **122**(3): p. 787-95.
46. Lambeth, J.D., T. Kawahara, and B. Diebold, *Regulation of Nox and Duox enzymatic activity and expression.* Free Radic Biol Med, 2007. **43**(3): p. 319-31.
47. Wright, H.L., et al., *Neutrophil function in inflammation and inflammatory diseases.* Rheumatology (Oxford), 2010. **49**(9): p. 1618-31.
48. Witko-Sarsat, V., et al., *Neutrophils: molecules, functions and pathophysiological aspects.* Lab Invest, 2000. **80**(5): p. 617-53.
49. Borregaard, N., O.E. Sorensen, and K. Theilgaard-Monch, *Neutrophil granules: a library of innate immunity proteins.* Trends Immunol, 2007. **28**(8): p. 340-5.
50. Faurschou, M. and N. Borregaard, *Neutrophil granules and secretory vesicles in inflammation.* Microbes Infect, 2003. **5**(14): p. 1317-27.
51. El-Benna, J., P.M. Dang, and M.A. Gougerot-Pocidalo, *Priming of the neutrophil NADPH oxidase activation: role of p47phox phosphorylation and NOX2 mobilization to the plasma membrane.* Semin Immunopathol, 2008. **30**(3): p. 279-89.
52. Akgul, C., D.A. Moulding, and S.W. Edwards, *Molecular control of neutrophil apoptosis.* FEBS Lett, 2001. **487**(3): p. 318-22.
53. Ward, C., et al., *Pharmacological manipulation of granulocyte apoptosis: potential therapeutic targets.* Trends Pharmacol Sci, 1999. **20**(12): p. 503-9.
54. D'Autreaux, B. and M.B. Toledano, *ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis.* Nat Rev Mol Cell Biol, 2007. **8**(10): p. 813-24.
55. Trachootham, D., J. Alexandre, and P. Huang, *Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?* Nat Rev Drug Discov, 2009. **8**(7): p. 579-91.

56. Bedard, K. and K.H. Krause, *The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology.* Physiol Rev, 2007. **87**(1): p. 245-313.
57. Lambeth, J.D., *NOX enzymes and the biology of reactive oxygen.* Nat Rev Immunol, 2004. **4**(3): p. 181-9.
58. Sareila, O., et al., *Identification of a region in p47phox/NCF1 crucial for phagocytic NADPH oxidase (NOX2) activation.* J Leukoc Biol, 2013. **93**(3): p. 427-35.
59. Sallusto, F., J. Geginat, and A. Lanzavecchia, *Central memory and effector memory T cell subsets: function, generation, and maintenance.* Annu Rev Immunol, 2004. **22**: p. 745-63.
60. Schmitt, T.M. and J.C. Zuniga-Pflucker, *Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro.* Immunity, 2002. **17**(6): p. 749-56.
61. Surh, C.D. and J. Sprent, *T-cell apoptosis detected in situ during positive and negative selection in the thymus.* Nature, 1994. **372**(6501): p. 100-3.
62. Shortman, K., et al., *The generation and fate of thymocytes.* Semin Immunol, 1990. **2**(1): p. 3-12.
63. Schatz, D.G., *V(D)J recombination.* Immunol Rev, 2004. **200**: p. 5-11.
64. Turner, S.J., et al., *Structural determinants of T-cell receptor bias in immunity.* Nat Rev Immunol, 2006. **6**(12): p. 883-94.
65. Benedict, C.L., et al., *Terminal deoxynucleotidyl transferase and repertoire development.* Immunological Reviews, 2000. **175**: p. 150-157.
66. Cabaniols, J.P., et al., *Most alpha/beta T cell receptor diversity is due to terminal deoxynucleotidyl transferase.* Journal of Experimental Medicine, 2001. **194**(9): p. 1385-1390.
67. Fugmann, S.D., et al., *The RAG proteins and V(D)J recombination: complexes, ends, and transposition.* Annu Rev Immunol, 2000. **18**: p. 495-527.
68. Oettinger, M.A., et al., *Rag-1 and Rag-2, Adjacent Genes That Synergistically Activate V(D)J Recombination.* Science, 1990. **248**(4962): p. 1517-1523.
69. Kawano, T., et al., *CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides.* Science, 1997. **278**(5343): p. 1626-9.
70. Shinkai, Y., et al., *RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement.* Cell, 1992. **68**(5): p. 855-67.
71. Brownlie, R.J. and R. Zamoyska, *T cell receptor signalling networks: branched, diversified and bounded.* Nat Rev Immunol, 2013. **13**(4): p. 257-69.
72. Clevers, H., et al., *The T cell receptor/CD3 complex: a dynamic protein ensemble.* Annu Rev Immunol, 1988. **6**: p. 629-62.
73. Rudolph, M.G., R.L. Stanfield, and I.A. Wilson, *How TCRs bind MHCs, peptides, and coreceptors.* Annu Rev Immunol, 2006. **24**: p. 419-66.
74. Kim, P.W., et al., *A zinc clasp structure tethers Lck to T cell coreceptors CD4 and CD8.* Science, 2003. **301**(5640): p. 1725-8.
75. van Oers, N.S., N. Killeen, and A. Weiss, *Lck regulates the tyrosine phosphorylation of the T cell receptor subunits and ZAP-70 in murine thymocytes.* J Exp Med, 1996. **183**(3): p. 1053-62.
76. Chan, A.C., et al., *The zeta chain is associated with a tyrosine kinase and upon T-cell antigen receptor stimulation associates with ZAP-70, a 70-kDa tyrosine phosphoprotein.* Proc Natl Acad Sci U S A, 1991. **88**(20): p. 9166-70.
77. Palacios, E.H. and A. Weiss, *Distinct roles for Syk and ZAP-70 during early thymocyte development.* J Exp Med, 2007. **204**(7): p. 1703-15.
78. Wang, H., et al., *ZAP-70: an essential kinase in T-cell signaling.* Cold Spring Harb Perspect Biol, 2010. **2**(5): p. a002279.
79. Elder, M.E., et al., *Human severe combined immunodeficiency due to a defect in ZAP-70, a T cell tyrosine kinase.* Science, 1994. **264**(5165): p. 1596-9.
80. Negishi, I., et al., *Essential Role for Zap-70 in Both Positive and Negative Selection of Thymocytes.* Nature, 1995. **376**(6539): p. 435-438.
81. Vivier, E., J.A. Nunes, and F. Vely, *Natural killer cell signaling pathways.* Science, 2004. **306**(5701): p. 1517-9.
82. Scielzo, C., et al., *ZAP-70 is expressed by normal and malignant human B-cell subsets of different maturational stage.* Leukemia, 2006. **20**(4): p. 689-95.
83. Chen, L., et al., *Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia.* Blood, 2002. **100**(13): p. 4609-14.

84. Takata, M., et al., *Tyrosine kinases Lyn and Syk regulate B cell receptor-coupled Ca2+ mobilization through distinct pathways.* EMBO J, 1994. **13**(6): p. 1341-9.
85. Colucci, F., et al., *Natural cytotoxicity uncoupled from the Syk and ZAP-70 intracellular kinases.* Nat Immunol, 2002. **3**(3): p. 288-94.
86. Starr, T.K., S.C. Jameson, and K.A. Hogquist, *Positive and negative selection of T cells.* Annu Rev Immunol, 2003. **21**: p. 139-76.
87. Klein, L., et al., *Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see).* Nat Rev Immunol, 2014. **14**(6): p. 377-91.
88. Germain, R.N., *T-cell development and the CD4-CD8 lineage decision.* Nat Rev Immunol, 2002. **2**(5): p. 309-22.
89. Klein, L., et al., *Antigen presentation in the thymus for positive selection and central tolerance induction.* Nat Rev Immunol, 2009. **9**(12): p. 833-44.
90. Palmer, E., *Negative selection--clearing out the bad apples from the T-cell repertoire.* Nat Rev Immunol, 2003. **3**(5): p. 383-91.
91. Kyewski, B. and L. Klein, *A central role for central tolerance.* Annu Rev Immunol, 2006. **24**: p. 571-606.
92. Anderson, M.S., et al., *Projection of an immunological self shadow within the thymus by the aire protein.* Science, 2002. **298**(5597): p. 1395-401.
93. Artyomov, M.N., et al., *CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery.* Proc Natl Acad Sci U S A, 2010. **107**(39): p. 16916-21.
94. Miceli, M.C. and J.R. Parnes, *The roles of CD4 and CD8 in T cell activation.* Semin Immunol, 1991. **3**(3): p. 133-41.
95. Chen, L. and D.B. Flies, *Molecular mechanisms of T cell co-stimulation and co-inhibition.* Nat Rev Immunol, 2013. **13**(4): p. 227-42.
96. Sharpe, A.H. and G.J. Freeman, *The B7-CD28 superfamily.* Nat Rev Immunol, 2002. **2**(2): p. 116-26.
97. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy.* Nat Rev Cancer, 2012. **12**(4): p. 252-64.
98. Curtsinger, J.M. and M.F. Mescher, *Inflammatory cytokines as a third signal for T cell activation.* Curr Opin Immunol, 2010. **22**(3): p. 333-40.
99. Curtsinger, J.M., et al., *Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells.* J Immunol, 1999. **162**(6): p. 3256-62.
100. Bromley, S.K., et al., *The immunological synapse and CD28-CD80 interactions.* Nat Immunol, 2001. **2**(12): p. 1159-66.
101. Grakoui, A., et al., *The immunological synapse: a molecular machine controlling T cell activation.* Science, 1999. **285**(5425): p. 221-7.
102. Huppa, J.B. and M.M. Davis, *T-cell-antigen recognition and the immunological synapse.* Nat Rev Immunol, 2003. **3**(12): p. 973-83.
103. Vogt, A.B., S. Spindeldreher, and H. Kropshofer, *Clustering of MHC-peptide complexes prior to their engagement in the immunological synapse: lipid raft and tetraspan microdomains.* Immunol Rev, 2002. **189**: p. 136-51.
104. Zou, W. and N.P. Restifo, *T(H)17 cells in tumour immunity and immunotherapy.* Nat Rev Immunol, 2010. **10**(4): p. 248-56.
105. Swain, S.L., K.K. McKinstry, and T.M. Strutt, *Expanding roles for CD4(+) T cells in immunity to viruses.* Nat Rev Immunol, 2012. **12**(2): p. 136-48.
106. Zhu, J. and W.E. Paul, *Heterogeneity and plasticity of T helper cells.* Cell Res, 2010. **20**(1): p. 4-12.
107. Szabo, S.J., et al., *Molecular mechanisms regulating Th1 immune responses.* Annu Rev Immunol, 2003. **21**: p. 713-58.
108. Romagnani, S., *The Th1/Th2 paradigm.* Immunol Today, 1997. **18**(6): p. 263-6.
109. Ansel, K.M., et al., *Regulation of Th2 differentiation and Il4 locus accessibility.* Annu Rev Immunol, 2006. **24**: p. 607-56.
110. Constant, S.L. and K. Bottomly, *Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches.* Annu Rev Immunol, 1997. **15**: p. 297-322.
111. Licona-Limon, P., et al., *TH2, allergy and group 2 innate lymphoid cells.* Nat Immunol, 2013. **14**(6): p. 536-42.

112. Redecke, V., et al., *Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma.* J Immunol, 2004. **172**(5): p. 2739-43.
113. Robinson, D.S., et al., *Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma.* N Engl J Med, 1992. **326**(5): p. 298-304.
114. Harrington, L.E., et al., *Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages.* Nat Immunol, 2005. **6**(11): p. 1123-32.
115. Park, H., et al., *A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17.* Nat Immunol, 2005. **6**(11): p. 1133-41.
116. Steinman, L., *A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage.* Nat Med, 2007. **13**(2): p. 139-45.
117. Dardalhon, V., et al., *Role of Th1 and Th17 cells in organ-specific autoimmunity.* J Autoimmun, 2008. **31**(3): p. 252-6.
118. Codarri, L., et al., *RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation.* Nat Immunol, 2011. **12**(6): p. 560-7.
119. Bettelli, E., et al., *Induction and effector functions of T(H)17 cells.* Nature, 2008. **453**(7198): p. 1051-7.
120. Stritesky, G.L., N. Yeh, and M.H. Kaplan, *IL-23 promotes maintenance but not commitment to the Th17 lineage.* J Immunol, 2008. **181**(9): p. 5948-55.
121. Pelletier, M., et al., *Evidence for a cross-talk between human neutrophils and Th17 cells.* Blood, 2010. **115**(2): p. 335-43.
122. Laan, M., et al., *Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways.* J Immunol, 1999. **162**(4): p. 2347-52.
123. Hori, S., T. Nomura, and S. Sakaguchi, *Control of regulatory T cell development by the transcription factor Foxp3.* Science, 2003. **299**(5609): p. 1057-61.
124. Fontenot, J.D., M.A. Gavin, and A.Y. Rudensky, *Foxp3 programs the development and function of CD4+CD25+ regulatory T cells.* Nat Immunol, 2003. **4**(4): p. 330-6.
125. Curotto de Lafaille, M.A. and J.J. Lafaille, *Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor?* Immunity, 2009. **30**(5): p. 626-35.
126. Wing, K. and S. Sakaguchi, *Regulatory T cells exert checks and balances on self tolerance and autoimmunity.* Nat Immunol, 2010. **11**(1): p. 7-13.
127. Sakaguchi, S., et al., *Regulatory T cells: how do they suppress immune responses?* Int Immunol, 2009. **21**(10): p. 1105-11.
128. Levine, A.G., et al., *Continuous requirement for the TCR in regulatory T cell function.* Nat Immunol, 2014. **15**(11): p. 1070-8.
129. Baumgart, D.C. and W.J. Sandborn, *Crohn's disease.* Lancet, 2012. **380**(9853): p. 1590-605.
130. Griffiths, C.E. and J.N. Barker, *Pathogenesis and clinical features of psoriasis.* Lancet, 2007. **370**(9583): p. 263-71.
131. Scott, D.L., F. Wolfe, and T.W. Huizinga, *Rheumatoid arthritis.* Lancet, 2010. **376**(9746): p. 1094-108.
132. Atkinson, M.A., G.S. Eisenbarth, and A.W. Michels, *Type 1 diabetes.* Lancet, 2014. **383**(9911): p. 69-82.
133. Compston, A. and A. Coles, *Multiple sclerosis.* Lancet, 2008. **372**(9648): p. 1502-17.
134. Nestle, F.O., D.H. Kaplan, and J. Barker, *Psoriasis.* N Engl J Med, 2009. **361**(5): p. 496-509.
135. Tsoi, L.C., et al., *Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity.* Nat Genet, 2012. **44**(12): p. 1341-8.
136. Genetic Analysis of Psoriasis, C., et al., *A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1.* Nat Genet, 2010. **42**(11): p. 985-90.
137. Chandran, V. and S.P. Raychaudhuri, *Geoepidemiology and environmental factors of psoriasis and psoriatic arthritis.* J Autoimmun, 2010. **34**(3): p. J314-21.
138. Gladman, D.D., et al., *Psoriatic arthritis: epidemiology, clinical features, course, and outcome.* Ann Rheum Dis, 2005. **64 Suppl 2**: p. ii14-7.
139. Alamanos, Y., P.V. Voulgari, and A.A. Drosos, *Incidence and prevalence of psoriatic arthritis: a systematic review.* J Rheumatol, 2008. **35**(7): p. 1354-8.
140. Langley, R.G., G.G. Krueger, and C.E. Griffiths, *Psoriasis: epidemiology, clinical features, and quality of life.* Ann Rheum Dis, 2005. **64 Suppl 2**: p. ii18-23; discussion ii24-5.
141. Christophers, E., *Psoriasis--epidemiology and clinical spectrum.* Clin Exp Dermatol, 2001. **26**(4): p. 314-20.

142. Henseler, T. and E. Christophers, *Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris.* J Am Acad Dermatol, 1985. **13**(3): p. 450-6.
143. Ferrandiz, C., et al., *Psoriasis of early and late onset: a clinical and epidemiologic study from Spain.* J Am Acad Dermatol, 2002. **46**(6): p. 867-73.
144. Bowcock, A.M. and J.N. Barker, *Genetics of psoriasis: the potential impact on new therapies.* J Am Acad Dermatol, 2003. **49**(2 Suppl): p. S51-6.
145. Abele, D.C., R.L. Dobson, and J.B. Graham, *Heredity and Psoriasis. Study of a Large Family.* Arch Dermatol, 1963. **88**: p. 38-47.
146. Duffy, D.L., L.S. Spelman, and N.G. Martin, *Psoriasis in Australian twins.* J Am Acad Dermatol, 1993. **29**(3): p. 428-34.
147. Lonnberg, A.S., et al., *Heritability of psoriasis in a large twin sample.* Br J Dermatol, 2013. **169**(2): p. 412-6.
148. Stuart, P.E., et al., *Genome-wide association analysis identifies three psoriasis susceptibility loci.* Nat Genet, 2010. **42**(11): p. 1000-4.
149. Roberson, E.D. and A.M. Bowcock, *Psoriasis genetics: breaking the barrier.* Trends Genet, 2010. **26**(9): p. 415-23.
150. Nair, R.P., et al., *Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C.* American Journal of Human Genetics, 2000. **66**(6): p. 1833-1844.
151. Nair, R.P., et al., *Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene.* American Journal of Human Genetics, 2006. **78**(5): p. 827-851.
152. Cargill, M., et al., *A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes.* American Journal of Human Genetics, 2007. **80**(2): p. 273-290.
153. Nair, R.P., et al., *Polymorphisms of the IL12B and IL23R genes are associated with psoriasis.* Journal of Investigative Dermatology, 2008. **128**(7): p. 1653-1661.
154. Johnston, A., et al., *Susceptibility-associated genetic variation at IL12B enhances Th1 polarization in psoriasis.* Human Molecular Genetics, 2013. **22**(9): p. 1807-1815.
155. Di Cesare, A., P. Di Meglio, and F.O. Nestle, *The IL-23/Th17 Axis in the Immunopathogenesis of Psoriasis.* Journal of Investigative Dermatology, 2009. **129**(6): p. 1339-1350.
156. Smith, R.L., et al., *Polymorphisms in the PTPN22 region are associated with psoriasis of early onset.* Br J Dermatol, 2008. **158**(5): p. 962-8.
157. Li, Y.H., et al., *Further Genetic Evidence for Three Psoriasis-Risk Genes: ADAM33, CDKAL1, and PTPN22.* Journal of Investigative Dermatology, 2009. **129**(3): p. 629-634.
158. Harper, E.G., et al., *Th17 Cytokines Stimulate CCL20 Expression in Keratinocytes In Vitro and In Vivo: Implications for Psoriasis Pathogenesis.* Journal of Investigative Dermatology, 2009. **129**(9): p. 2175-2183.
159. Mckay, I.A. and I.M. Leigh, *Altered Keratinocyte Growth and Differentiation in Psoriasis.* Clinics in Dermatology, 1995. **13**(2): p. 105-&.
160. Nickoloff, B.J. and Y. Naidu, *Perturbation of Epidermal Barrier Function Correlates with Initiation of Cytokine Cascade in Human Skin.* Journal of the American Academy of Dermatology, 1994. **30**(4): p. 535-546.
161. Pinkus, H. and A.H. Mehregan, *Primary Histologic Lesion of Seborrheic Dermatitis and Psoriasis.* Journal of Investigative Dermatology, 1966. **46**(1): p. 109-&.
162. Leigh, I.M., et al., *Keratins (K16 and K17) as Markers of Keratinocyte Hyperproliferation in Psoriasis in-Vivo and in-Vitro.* British Journal of Dermatology, 1995. **133**(4): p. 501-511.
163. Truzzi, F., et al., *A novel human psoriatic skin reconstruct: The role of transit amplifying keratinocytes and of p75 neurotrophin receptor.* Journal of Investigative Dermatology, 2012. **132**: p. S53-S53.
164. Sabat, R., et al., *Immunopathogenesis of psoriasis.* Experimental Dermatology, 2007. **16**(10): p. 779-798.
165. Kryczek, I., et al., *Induction of IL-17(+) T cell trafficking and development by IFN-gamma: Mechanism and pathological relevance in psoriasis.* Journal of Immunology, 2008. **181**(7): p. 4733-4741.
166. Boyman, O., et al., *Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha.* Journal of Experimental Medicine, 2004. **199**(5): p. 731-736.

167. Nickoloff, B.J., et al., *Severe Combined Immunodeficiency Mouse and Human Psoriatic Skin Chimeras - Validation of a New Animal-Model.* American Journal of Pathology, 1995. **146**(3): p. 580-588.
168. Lebwohl, M., et al., *A novel targeted T-cell modulator, efalizumab, for plaque psoriasis.* New England Journal of Medicine, 2003. **349**(21): p. 2004-2013.
169. Ellis, C.N., et al., *Cyclosporine Improves Psoriasis in a Double-Blind-Study.* Jama-Journal of the American Medical Association, 1986. **256**(22): p. 3110-3116.
170. Schon, M., et al., *Critical role of neutrophils for the generation of psoriasiform skin lesions in flaky skin mice.* Journal of Investigative Dermatology, 2000. **114**(5): p. 976-983.
171. Toichi, E., T. Tachibana, and F. Furukawa, *Rapid improvement of psoriasis vulgaris during drug-induced agranulocytosis.* Journal of the American Academy of Dermatology, 2000. **43**(2): p. 391-395.
172. Heidenreich, R., M. Rocken, and K. Ghoreschi, *Angiogenesis drives psoriasis pathogenesis.* International Journal of Experimental Pathology, 2009. **90**(3): p. 232-248.
173. Guerard, S. and R. Pouliot, *The role of angiogenesis in the pathogenesis of psoriasis: mechanisms and clinical implications.* Journal of Clinical Dermatology Research, 2012. **S4**: p. 007.
174. Henno, A., et al., *Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis.* British Journal of Dermatology, 2009. **160**(3): p. 581-590.
175. Zhang, Y., H. Matsuo, and E. Morita, *Vascular endothelial growth factor 121 is the predominant isoform in psoriatic scales.* Experimental Dermatology, 2005. **14**(10): p. 758-764.
176. Detmar, M., et al., *Overexpression of Vascular-Permeability Factor Vascular Endothelial Growth-Factor and Its Receptors in Psoriasis.* Journal of Experimental Medicine, 1994. **180**(3): p. 1141-1146.
177. Detmar, M., et al., *Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice.* Journal of Investigative Dermatology, 1998. **111**(1): p. 1-6.
178. Lowes, M.A., A.M. Bowcock, and J.G. Krueger, *Pathogenesis and therapy of psoriasis.* Nature, 2007. **445**(7130): p. 866-73.
179. Gulliver, W., *Long-term prognosis in patients with psoriasis.* Br J Dermatol, 2008. **159 Suppl 2**: p. 2-9.
180. Choi, J. and J.Y. Koo, *Quality of life issues in psoriasis.* J Am Acad Dermatol, 2003. **49**(2 Suppl): p. S57-61.
181. Chaudhari, U., et al., *Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial.* Lancet, 2001. **357**(9271): p. 1842-7.
182. Leonardi, C.L., et al., *Etanercept as monotherapy in patients with psoriasis.* N Engl J Med, 2003. **349**(21): p. 2014-22.
183. Langley, R.G., et al., *Secukinumab in plaque psoriasis--results of two phase 3 trials.* N Engl J Med, 2014. **371**(4): p. 326-38.
184. Papp, K.A., et al., *Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2).* Lancet, 2008. **371**(9625): p. 1675-84.
185. Centers for Disease, C. and Prevention, *National and state medical expenditures and lost earnings attributable to arthritis and other rheumatic conditions--United States, 2003.* MMWR Morb Mortal Wkly Rep, 2007. **56**(1): p. 4-7.
186. Salaffi, F., et al., *The health-related quality of life in rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis: a comparison with a selected sample of healthy people.* Health Qual Life Outcomes, 2009. **7**: p. 25.
187. Zhang, W. and A.H. Anis, *The economic burden of rheumatoid arthritis: beyond health care costs.* Clin Rheumatol, 2011. **30 Suppl 1**: p. S25-32.
188. Stahl, E.A., et al., *Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci.* Nat Genet, 2010. **42**(6): p. 508-14.
189. Okada, Y., et al., *Genetics of rheumatoid arthritis contributes to biology and drug discovery.* Nature, 2014. **506**(7488): p. 376-81.
190. McInnes, I.B. and G. Schett, *The pathogenesis of rheumatoid arthritis.* N Engl J Med, 2011. **365**(23): p. 2205-19.
191. Aletaha, D., et al., *2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.* Ann Rheum Dis, 2010. **69**(9): p. 1580-8.
192. Schellekens, G.A., et al., *The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.* Arthritis Rheum, 2000. **43**(1): p. 155-63.

193. Schellekens, G.A., et al., *Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies.* J Clin Invest, 1998. **101**(1): p. 273-81.
194. Rantapaa-Dahlqvist, S., et al., *Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis.* Arthritis Rheum, 2003. **48**(10): p. 2741-9.
195. Nielen, M.M., et al., *Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors.* Arthritis Rheum, 2004. **50**(2): p. 380-6.
196. Kroot, E.J., et al., *The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis.* Arthritis Rheum, 2000. **43**(8): p. 1831-5.
197. Meyer, O., et al., *Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage.* Ann Rheum Dis, 2003. **62**(2): p. 120-6.
198. Kuhn, K.A., et al., *Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis.* J Clin Invest, 2006. **116**(4): p. 961-73.
199. Hill, J.A., et al., *Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice.* J Exp Med, 2008. **205**(4): p. 967-79.
200. Haag, S., et al., *Human alpha-enolase is immunogenic, but not arthritogenic, in HLA-DR4-transgenic mice: comment on the article by Kinloch et al.* Arthritis Rheum, 2012. **64**(5): p. 1689-91; author reply 1691-2.
201. Padyukov, L., et al., *A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis.* Ann Rheum Dis, 2011. **70**(2): p. 259-65.
202. van der Woude, D., et al., *Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis.* Arthritis Rheum, 2009. **60**(4): p. 916-23.
203. Daha, N.A. and R.E. Toes, *Rheumatoid arthritis: Are ACPA-positive and ACPA-negative RA the same disease?* Nat Rev Rheumatol, 2011. **7**(4): p. 202-3.
204. Alamanos, Y. and A.A. Drosos, *Epidemiology of adult rheumatoid arthritis.* Autoimmun Rev, 2005. **4**(3): p. 130-6.
205. Ferucci, E.D., D.W. Templin, and A.P. Lanier, *Rheumatoid arthritis in American Indians and Alaska Natives: a review of the literature.* Semin Arthritis Rheum, 2005. **34**(4): p. 662-7.
206. Kvien, T.K., et al., *Epidemiological aspects of rheumatoid arthritis: the sex ratio.* Ann N Y Acad Sci, 2006. **1069**: p. 212-22.
207. Goemaere, S., et al., *Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition.* J Rheumatol, 1990. **17**(12): p. 1620-2.
208. van der Woude, D., et al., *Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis.* Ann Rheum Dis, 2010. **69**(8): p. 1554-61.
209. Brink, M., et al., *Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis.* Arthritis Rheum, 2013. **65**(4): p. 899-910.
210. MacGregor, A.J., et al., *Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins.* Arthritis Rheum, 2000. **43**(1): p. 30-7.
211. Silman, A.J., et al., *Twin concordance rates for rheumatoid arthritis: results from a nationwide study.* Br J Rheumatol, 1993. **32**(10): p. 903-7.
212. Aho, K., et al., *Occurrence of rheumatoid arthritis in a nationwide series of twins.* J Rheumatol, 1986. **13**(5): p. 899-902.
213. Okada, Y., et al., *Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population.* Nat Genet, 2012. **44**(5): p. 511-6.
214. Weyand, C.M., et al., *The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis.* Ann Intern Med, 1992. **117**(10): p. 801-6.
215. Holoshitz, J., *The rheumatoid arthritis HLA-DRB1 shared epitope.* Curr Opin Rheumatol, 2010. **22**(3): p. 293-8.
216. Gregersen, P.K., J. Silver, and R.J. Winchester, *The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis.* Arthritis Rheum, 1987. **30**(11): p. 1205-13.
217. Huizinga, T.W., et al., *Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins.* Arthritis Rheum, 2005. **52**(11): p. 3433-8.

218. van der Helm-van Mil, A.H., et al., *The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis.* Arthritis Rheum, 2006. **54**(4): p. 1117-21.
219. van Gaalen, F.A., et al., *Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis.* Arthritis Rheum, 2004. **50**(7): p. 2113-21.
220. Hill, J.A., et al., *Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule.* J Immunol, 2003. **171**(2): p. 538-41.
221. Viatte, S., D. Plant, and S. Raychaudhuri, *Genetics and epigenetics of rheumatoid arthritis.* Nat Rev Rheumatol, 2013. **9**(3): p. 141-53.
222. Rieck, M., et al., *Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes.* J Immunol, 2007. **179**(7): p. 4704-10.
223. Stanford, S.M. and N. Bottini, *PTPN22: the archetypal non-HLA autoimmunity gene.* Nat Rev Rheumatol, 2014. **10**(10): p. 602-11.
224. Gregersen, P.K., *Gaining insight into PTPN22 and autoimmunity.* Nat Genet, 2005. **37**(12): p. 1300-2.
225. Vang, T., et al., *Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant.* Nat Genet, 2005. **37**(12): p. 1317-9.
226. Lee, Y.H., et al., *The PTPN22 C1858T functional polymorphism and autoimmune diseases--a meta-analysis.* Rheumatology (Oxford), 2007. **46**(1): p. 49-56.
227. Gregersen, P.K., et al., *PTPN22: setting thresholds for autoimmunity.* Semin Immunol, 2006. **18**(4): p. 214-23.
228. Menard, L., et al., *The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans.* J Clin Invest, 2011. **121**(9): p. 3635-44.
229. Habib, T., et al., *Altered B cell homeostasis is associated with type I diabetes and carriers of the PTPN22 allelic variant.* J Immunol, 2012. **188**(1): p. 487-96.
230. Arechiga, A.F., et al., *Cutting edge: the PTPN22 allelic variant associated with autoimmunity impairs B cell signaling.* J Immunol, 2009. **182**(6): p. 3343-7.
231. Bottini, N., et al., *A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes.* Nat Genet, 2004. **36**(4): p. 337-8.
232. Begovich, A.B., et al., *A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis.* Am J Hum Genet, 2004. **75**(2): p. 330-7.
233. Kyogoku, C., et al., *Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE.* Am J Hum Genet, 2004. **75**(3): p. 504-7.
234. Velaga, M.R., et al., *The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease.* J Clin Endocrinol Metab, 2004. **89**(11): p. 5862-5.
235. Heliovaara, M., et al., *Smoking and Risk of Rheumatoid-Arthritis.* Journal of Rheumatology, 1993. **20**(11): p. 1830-1835.
236. Vessey, M.P., L. Villard-Mackintosh, and D. Yeates, *Oral contraceptives, cigarette smoking and other factors in relation to arthritis.* Contraception, 1987. **35**(5): p. 457-64.
237. Uhlig, T., K.B. Hagen, and T.K. Kvien, *Current tobacco smoking, formal education, and the risk of rheumatoid arthritis.* J Rheumatol, 1999. **26**(1): p. 47-54.
238. Karlson, E.W., et al., *A retrospective cohort study of cigarette smoking and risk of rheumatoid arthritis in female health professionals.* Arthritis Rheum, 1999. **42**(5): p. 910-7.
239. Silman, A.J., J. Newman, and A.J. MacGregor, *Cigarette smoking increases the risk of rheumatoid arthritis - Results from a nationwide study of disease-discordant twins.* Arthritis and Rheumatism, 1996. **39**(5): p. 732-735.
240. Wolfe, F., *The effect of smoking on clinical, laboratory, and radiographic status in rheumatoid arthritis.* J Rheumatol, 2000. **27**(3): p. 630-7.
241. Saag, K.G., et al., *Cigarette smoking and rheumatoid arthritis severity.* Ann Rheum Dis, 1997. **56**(8): p. 463-9.
242. Padyukov, L., et al., *A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis.* Arthritis Rheum, 2004. **50**(10): p. 3085-92.

243. Linn-Rasker, S.P., et al., *Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles.* Ann Rheum Dis, 2006. **65**(3): p. 366-71.
244. Klareskog, L., et al., *A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination.* Arthritis Rheum, 2006. **54**(1): p. 38-46.
245. Makrygiannakis, D., et al., *Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells.* Ann Rheum Dis, 2008. **67**(10): p. 1488-92.
246. Willemze, A., et al., *The interaction between HLA shared epitope alleles and smoking and its contribution to autoimmunity against several citrullinated antigens.* Arthritis Rheum, 2011. **63**(7): p. 1823-32.
247. Kallberg, H., et al., *Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis.* Am J Hum Genet, 2007. **80**(5): p. 867-75.
248. Lee, H.S., et al., *Interaction between smoking, the shared epitope, and anti-cyclic citrullinated peptide: a mixed picture in three large North American rheumatoid arthritis cohorts.* Arthritis Rheum, 2007. **56**(6): p. 1745-53.
249. Hensvold, A.H., et al., *Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins.* Ann Rheum Dis, 2015. **74**(2): p. 375-80.
250. Silman, A., A. Kay, and P. Brennan, *Timing of pregnancy in relation to the onset of rheumatoid arthritis.* Arthritis Rheum, 1992. **35**(2): p. 152-5.
251. Nelson, J.L. and M. Ostensen, *Pregnancy and rheumatoid arthritis.* Rheum Dis Clin North Am, 1997. **23**(1): p. 195-212.
252. Wilder, R.L., *Hormones, pregnancy, and autoimmune diseases.* Ann N Y Acad Sci, 1998. **840**: p. 45-50.
253. Spector, T.D. and M.C. Hochberg, *The protective effect of the oral contraceptive pill on rheumatoid arthritis: an overview of the analytic epidemiological studies using meta-analysis.* J Clin Epidemiol, 1990. **43**(11): p. 1221-30.
254. van Zeben, D., et al., *Diminished incidence of severe rheumatoid arthritis associated with oral contraceptive use.* Arthritis Rheum, 1990. **33**(10): p. 1462-5.
255. Vandenbroucke, J.P., et al., *Oral contraceptives and rheumatoid arthritis: further evidence for a preventive effect.* Lancet, 1982. **2**(8303): p. 839-42.
256. Jansson, L., T. Olsson, and R. Holmdahl, *Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice.* J Neuroimmunol, 1994. **53**(2): p. 203-7.
257. Josefsson, E. and A. Tarkowski, *Suppression of type II collagen-induced arthritis by the endogenous estrogen metabolite 2-methoxyestradiol.* Arthritis Rheum, 1997. **40**(1): p. 154-63.
258. Holmdahl, R., L. Jansson, and M. Andersson, *Female sex hormones suppress development of collagen-induced arthritis in mice.* Arthritis Rheum, 1986. **29**(12): p. 1501-9.
259. Hall, G.M., et al., *A randomised controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal rheumatoid arthritis.* Ann Rheum Dis, 1994. **53**(2): p. 112-6.
260. D'Elia, H.F., et al., *Influence of hormone replacement therapy on disease progression and bone mineral density in rheumatoid arthritis.* J Rheumatol, 2003. **30**(7): p. 1456-63.
261. Ansar Ahmed, S., W.J. Penhale, and N. Talal, *Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action.* Am J Pathol, 1985. **121**(3): p. 531-51.
262. Jansson, L. and R. Holmdahl, *Estrogen-mediated immunosuppression in autoimmune diseases.* Inflamm Res, 1998. **47**(7): p. 290-301.
263. Polanczyk, M.J., et al., *Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment.* J Immunol, 2004. **173**(4): p. 2227-30.
264. Beral, V., *Breast cancer and hormone-replacement therapy in the Million Women Study.* Lancet, 2003. **362**(9382): p. 419-27.
265. Wucherpfennig, K.W. and J.L. Strominger, *Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein.* Cell, 1995. **80**(5): p. 695-705.
266. Albani, S. and D.A. Carson, *A multistep molecular mimicry hypothesis for the pathogenesis of rheumatoid arthritis.* Immunol Today, 1996. **17**(10): p. 466-70.

267. Cole, B.C. and M.M. Griffiths, *Triggering and exacerbation of autoimmune arthritis by the Mycoplasma arthritidis superantigen MAM.* Arthritis Rheum, 1993. **36**(7): p. 994-1002.
268. Benoist, C. and D. Mathis, *Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry?* Nat Immunol, 2001. **2**(9): p. 797-801.
269. Ogrendik, M., *Rheumatoid arthritis is linked to oral bacteria: etiological association.* Mod Rheumatol, 2009. **19**(5): p. 453-6.
270. Rosenstein, E.D., et al., *Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis.* Inflammation, 2004. **28**(6): p. 311-8.
271. Kharlamova, N., et al., *Antibodies to Porphyromonas gingivalis Indicate Interaction Between Oral Infection, Smoking, and Risk Genes in Rheumatoid Arthritis Etiology.* Arthritis Rheumatol, 2016. **68**(3): p. 604-13.
272. Hitchon, C.A., et al., *Antibodies to porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives.* J Rheumatol, 2010. **37**(6): p. 1105-12.
273. Singh, J.A., et al., *2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis.* Arthritis Rheumatol, 2016. **68**(1): p. 1-26.
274. Cutolo, M., et al., *Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis.* Ann Rheum Dis, 2001. **60**(8): p. 729-35.
275. Segal, R., M. Yaron, and B. Tartakovsky, *Methotrexate: mechanism of action in rheumatoid arthritis.* Semin Arthritis Rheum, 1990. **20**(3): p. 190-200.
276. Taylor, P.C. and M. Feldmann, *Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis.* Nat Rev Rheumatol, 2009. **5**(10): p. 578-82.
277. Lipsky, P.E., et al., *Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group.* N Engl J Med, 2000. **343**(22): p. 1594-602.
278. Aggarwal, B.B., S.C. Gupta, and J.H. Kim, *Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey.* Blood, 2012. **119**(3): p. 651-65.
279. Hanauer, S.B., et al., *Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial.* Lancet, 2002. **359**(9317): p. 1541-9.
280. Wolfe, F. and K. Michaud, *Biologic treatment of rheumatoid arthritis and the risk of malignancy: analyses from a large US observational study.* Arthritis Rheum, 2007. **56**(9): p. 2886-95.
281. Askling, J., et al., *Cancer risk in patients with rheumatoid arthritis treated with anti-tumor necrosis factor alpha therapies: does the risk change with the time since start of treatment?* Arthritis Rheum, 2009. **60**(11): p. 3180-9.
282. Mariette, X., et al., *Malignancies associated with tumour necrosis factor inhibitors in registries and prospective observational studies: a systematic review and meta-analysis.* Ann Rheum Dis, 2011. **70**(11): p. 1895-904.
283. Kremer, J.M., et al., *Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig.* N Engl J Med, 2003. **349**(20): p. 1907-15.
284. Genovese, M.C., et al., *Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition.* N Engl J Med, 2005. **353**(11): p. 1114-23.
285. Schiff, M., et al., *Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: a phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate.* Ann Rheum Dis, 2008. **67**(8): p. 1096-103.
286. Smolen, J.S., et al., *Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial.* Lancet, 2008. **371**(9617): p. 987-97.
287. Hirano, T., et al., *Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis.* Eur J Immunol, 1988. **18**(11): p. 1797-801.
288. Houssiau, F.A., et al., *Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides.* Arthritis Rheum, 1988. **31**(6): p. 784-8.
289. Emery, P., et al., *IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial.* Ann Rheum Dis, 2008. **67**(11): p. 1516-23.

290. Zhou, L., et al., *IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways.* Nat Immunol, 2007. **8**(9): p. 967-74.
291. McGeachy, M.J., et al., *TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology.* Nat Immunol, 2007. **8**(12): p. 1390-7.
292. Yen, D., et al., *IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6.* J Clin Invest, 2006. **116**(5): p. 1310-6.
293. Eugster, H.P., et al., *IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis.* Eur J Immunol, 1998. **28**(7): p. 2178-87.
294. Sims, J.E. and D.E. Smith, *The IL-1 family: regulators of immunity.* Nat Rev Immunol, 2010. **10**(2): p. 89-102.
295. Cohen, S., et al., *Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial.* Arthritis Rheum, 2002. **46**(3): p. 614-24.
296. Joosten, L.A., et al., *IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation.* J Immunol, 1999. **163**(9): p. 5049-55.
297. Abramson, S.B. and A. Amin, *Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage.* Rheumatology (Oxford), 2002. **41**(9): p. 972-80.
298. Singh, J.A., et al., *A network meta-analysis of randomized controlled trials of biologics for rheumatoid arthritis: a Cochrane overview.* CMAJ, 2009. **181**(11): p. 787-96.
299. Singh, J.A., et al., *Biologics for rheumatoid arthritis: an overview of Cochrane reviews.* Sao Paulo Med J, 2010. **128**(5): p. 309-10.
300. Cohen, S.B., et al., *Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks.* Arthritis Rheum, 2006. **54**(9): p. 2793-806.
301. Edwards, J.C., et al., *Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis.* N Engl J Med, 2004. **350**(25): p. 2572-81.
302. McLaughlin, P., et al., *Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program.* J Clin Oncol, 1998. **16**(8): p. 2825-33.
303. Coiffier, B., et al., *CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma.* N Engl J Med, 2002. **346**(4): p. 235-42.
304. Gurcan, H.M., et al., *A review of the current use of rituximab in autoimmune diseases.* Int Immunopharmacol, 2009. **9**(1): p. 10-25.
305. Hawker, K., et al., *Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial.* Ann Neurol, 2009. **66**(4): p. 460-71.
306. Looney, R.J., et al., *B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab.* Arthritis Rheum, 2004. **50**(8): p. 2580-9.
307. Hegen, M., et al., *Utility of animal models for identification of potential therapeutics for rheumatoid arthritis.* Ann Rheum Dis, 2008. **67**(11): p. 1505-15.
308. Kollias, G., et al., *Animal models for arthritis: innovative tools for prevention and treatment.* Ann Rheum Dis, 2011. **70**(8): p. 1357-62.
309. Brand, D.D., K.A. Latham, and E.F. Rosloniec, *Collagen-induced arthritis.* Nat Protoc, 2007. **2**(5): p. 1269-75.
310. Holmdahl, R., et al., *Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis.* Immunol Rev, 2001. **184**: p. 184-202.
311. Durie, F.H., R.A. Fava, and R.J. Noelle, *Collagen-induced arthritis as a model of rheumatoid arthritis.* Clin Immunol Immunopathol, 1994. **73**(1): p. 11-8.
312. Holmdahl, R., et al., *The molecular pathogenesis of collagen-induced arthritis in mice--a model for rheumatoid arthritis.* Ageing Res Rev, 2002. **1**(1): p. 135-47.
313. Wooley, P.H., et al., *Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates.* J Exp Med, 1981. **154**(3): p. 688-700.
314. Corthay, A., et al., *Collagen-induced arthritis development requires alpha beta T cells but not gamma delta T cells: studies with T cell-deficient (TCR mutant) mice.* Int Immunol, 1999. **11**(7): p. 1065-73.

315. Michaelsson, E., et al., *Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection.* Eur J Immunol, 1992. **22**(7): p. 1819-25.
316. Corthay, A., et al., *Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis.* Eur J Immunol, 1998. **28**(8): p. 2580-90.
317. Corthay, A., J. Backlund, and R. Holmdahl, *Role of glycopeptide-specific T cells in collagen-induced arthritis: an example how post-translational modification of proteins may be involved in autoimmune disease.* Ann Med, 2001. **33**(7): p. 456-65.
318. Holmdahl, R., et al., *Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II.* Clin Exp Immunol, 1985. **62**(3): p. 639-46.
319. Svensson, L., et al., *B cell-deficient mice do not develop type II collagen-induced arthritis (CIA).* Clin Exp Immunol, 1998. **111**(3): p. 521-6.
320. Stuart, J.M. and F.J. Dixon, *Serum transfer of collagen-induced arthritis in mice.* J Exp Med, 1983. **158**(2): p. 378-92.
321. Alonzi, T., et al., *Interleukin 6 is required for the development of collagen-induced arthritis.* J Exp Med, 1998. **187**(4): p. 461-8.
322. van den Berg, W.B., et al., *Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment.* Clin Exp Immunol, 1994. **95**(2): p. 237-43.
323. Williams, R.O., M. Feldmann, and R.N. Maini, *Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis.* Proc Natl Acad Sci U S A, 1992. **89**(20): p. 9784-8.
324. Webb, L.M., M.J. Walmsley, and M. Feldmann, *Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 co-stimulatory pathway: requirement for both B7-1 and B7-2.* Eur J Immunol, 1996. **26**(10): p. 2320-8.
325. Rowley, M.J., K.S. Nandakumar, and R. Holmdahl, *The role of collagen antibodies in mediating arthritis.* Mod Rheumatol, 2008. **18**(5): p. 429-41.
326. Nandakumar, K.S., L. Svensson, and R. Holmdahl, *Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes.* Am J Pathol, 2003. **163**(5): p. 1827-37.
327. Kagari, T., et al., *Essential role of Fc gamma receptors in anti-type II collagen antibody-induced arthritis.* J Immunol, 2003. **170**(8): p. 4318-24.
328. Tanaka, D., et al., *Essential role of neutrophils in anti-type II collagen antibody and lipopolysaccharide-induced arthritis.* Immunology, 2006. **119**(2): p. 195-202.
329. Nandakumar, K.S. and R. Holmdahl, *Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis.* Arthritis Res Ther, 2006. **8**(6): p. 223.
330. Hietala, M.A., et al., *Complement activation by both classical and alternative pathways is critical for the effector phase of arthritis.* Eur J Immunol, 2004. **34**(4): p. 1208-16.
331. Kagari, T., H. Doi, and T. Shimozato, *The importance of IL-1 beta and TNF-alpha, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis.* J Immunol, 2002. **169**(3): p. 1459-66.
332. Khachigian, L.M., *Collagen antibody-induced arthritis.* Nat Protoc, 2006. **1**(5): p. 2512-6.
333. Ditzel, H.J., *The K/BxN mouse: a model of human inflammatory arthritis.* Trends Mol Med, 2004. **10**(1): p. 40-5.
334. Iwanami, K., et al., *Crucial role of the interleukin-6/interleukin-17 cytokine axis in the induction of arthritis by glucose-6-phosphate isomerase.* Arthritis Rheum, 2008. **58**(3): p. 754-63.
335. Bockermann, R., et al., *Induction of a B-cell-dependent chronic arthritis with glucose-6-phosphate isomerase.* Arthritis Res Ther, 2005. **7**(6): p. R1316-24.
336. Wipke, B.T. and P.M. Allen, *Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis.* J Immunol, 2001. **167**(3): p. 1601-8.
337. Chen, M., et al., *Neutrophil-derived leukotriene B4 is required for inflammatory arthritis.* J Exp Med, 2006. **203**(4): p. 837-42.
338. Ji, H., et al., *Arthritis critically dependent on innate immune system players.* Immunity, 2002. **16**(2): p. 157-68.

339. Vingsbo, C., et al., *Pristane-induced arthritis in rats: a new model for rheumatoid arthritis with a chronic disease course influenced by both major histocompatibility complex and non-major histocompatibility complex genes.* Am J Pathol, 1996. **149**(5): p. 1675-83.
340. Tuncel, J., et al., *Animal Models of Rheumatoid Arthritis (I): Pristane-Induced Arthritis in the Rat.* PLoS One, 2016. **11**(5): p. e0155936.
341. Stasiuk, L.M., et al., *Pristane-induced arthritis is CD4+ T-cell dependent.* Immunology, 1997. **90**(1): p. 81-6.
342. Holmberg, J., et al., *Pristane, a non-antigenic adjuvant, induces MHC class II-restricted, arthritogenic T cells in the rat.* J Immunol, 2006. **176**(2): p. 1172-9.
343. Sakaguchi, N., et al., *Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice.* Nature, 2003. **426**(6965): p. 454-60.
344. Tanaka, S., et al., *Graded attenuation of TCR signaling elicits distinct autoimmune diseases by altering thymic T cell selection and regulatory T cell function.* J Immunol, 2010. **185**(4): p. 2295-305.
345. Nakamura, T., et al., *Induction of autoimmune arthritis after direct injection of bone marrow cells from arthritis-prone SKG/Jcl mice into bone cavity of normal mice.* Biol Pharm Bull, 2014. **37**(11): p. 1719-26.
346. Hsu, L.Y., et al., *A hypomorphic allele of ZAP-70 reveals a distinct thymic threshold for autoimmune disease versus autoimmune reactivity.* J Exp Med, 2009. **206**(11): p. 2527-41.
347. Zhao, W., et al., *Analysis of accumulating clonotypes of T cell in joints of a spontaneous murine model of rheumatoid arthritis.* Cell Mol Immunol, 2004. **1**(4): p. 300-3.
348. Wakasa-Morimoto, C., et al., *Arthritis and pneumonitis produced by the same T cell clones from mice with spontaneous autoimmune arthritis.* Int Immunol, 2008. **20**(10): p. 1331-42.
349. Ito, Y., et al., *Detection of T cell responses to a ubiquitous cellular protein in autoimmune disease.* Science, 2014. **346**(6207): p. 363-8.
350. Edwards, C.J. and C. Cooper, *Early environmental factors and rheumatoid arthritis.* Clin Exp Immunol, 2006. **143**(1): p. 1-5.
351. Yoshitomi, H., et al., *A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice.* J Exp Med, 2005. **201**(6): p. 949-60.
352. Hashimoto, M., et al., *Complement drives Th17 cell differentiation and triggers autoimmune arthritis.* J Exp Med, 2010. **207**(6): p. 1135-43.
353. Hida, S., et al., *Cell wall beta-glucan derived from Candida albicans acts as a trigger for autoimmune arthritis in SKG mice.* Biol Pharm Bull, 2007. **30**(8): p. 1589-92.
354. Ruutu, M., et al., *beta-glucan triggers spondylarthritis and Crohn's disease-like ileitis in SKG mice.* Arthritis Rheum, 2012. **64**(7): p. 2211-22.
355. Cutolo, M., et al., *Sex hormones and rheumatoid arthritis.* Autoimmun Rev, 2002. **1**(5): p. 284-9.
356. Lee, E.J., et al., *Investigation of the relationship between the onset of arthritis and uveitis in genetically predisposed SKG mice.* Arthritis Res Ther, 2015. **17**: p. 218.
357. Keith, R.C., et al., *Testosterone is protective in the sexually dimorphic development of arthritis and lung disease in SKG mice.* Arthritis Rheum, 2013. **65**(6): p. 1487-93.
358. Shaw, L.A., et al., *Pregnancy amelioration of arthritis in SKG mice corresponds with alterations in serum amyloid A3 levels.* Am J Clin Exp Immunol, 2012. **1**(1): p. 12-19.
359. Ostensen, M., *Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus.* Ann N Y Acad Sci, 1999. **876**: p. 131-43; discussion 144.
360. Inoue, K., E. Inoue, and Y. Imai, *Female sex hormones ameliorate arthritis in SKG mice.* Biochem Biophys Res Commun, 2013. **434**(4): p. 740-5.
361. Caetano-Lopes, J., et al., *Chronic arthritis leads to disturbances in the bone collagen network.* Arthritis Res Ther, 2010. **12**(1): p. R9.
362. Caetano-Lopes, J., et al., *Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties.* Clin Exp Rheumatol, 2009. **27**(3): p. 475-82.
363. Keller, K.K., et al., *Bone formation and resorption are both increased in experimental autoimmune arthritis.* PLoS One, 2012. **7**(12): p. e53034.
364. Charles, J.F., et al., *Inflammatory arthritis increases mouse osteoclast precursors with myeloid suppressor function.* J Clin Invest, 2012. **122**(12): p. 4592-605.

365. Keller, K.K., et al., *Systemic but no local effects of combined zoledronate and parathyroid hormone treatment in experimental autoimmune arthritis.* PLoS One, 2014. **9**(3): p. e92359.
366. Keller, K.K., et al., *SKG arthritis as a model for evaluating therapies in rheumatoid arthritis with special focus on bone changes.* Rheumatol Int, 2013. **33**(5): p. 1127-33.
367. Rehaume, L.M., et al., *ZAP-70 genotype disrupts the relationship between microbiota and host, leading to spondyloarthritis and ileitis in SKG mice.* Arthritis Rheumatol, 2014. **66**(10): p. 2780-92.
368. Benham, H., et al., *Interleukin-23 mediates the intestinal response to microbial beta-1,3-glucan and the development of spondyloarthritis pathology in SKG mice.* Arthritis Rheumatol, 2014. **66**(7): p. 1755-67.
369. Shiomi, A., et al., *GM-CSF but not IL-17 is critical for the development of severe interstitial lung disease in SKG mice.* J Immunol, 2014. **193**(2): p. 849-59.
370. Keith, R.C., et al., *A novel model of rheumatoid arthritis-associated interstitial lung disease in SKG mice.* Exp Lung Res, 2012. **38**(2): p. 55-66.
371. Khmaladze, I., et al., *Mannan induces ROS-regulated, IL-17A-dependent psoriasis arthritis-like disease in mice.* Proc Natl Acad Sci U S A, 2014. **111**(35): p. E3669-78.
372. Choy, E.H. and G.S. Panayi, *Cytokine pathways and joint inflammation in rheumatoid arthritis.* N Engl J Med, 2001. **344**(12): p. 907-16.
373. Feldmann, M., *Development of anti-TNF therapy for rheumatoid arthritis.* Nat Rev Immunol, 2002. **2**(5): p. 364-71.
374. Hata, H., et al., *Distinct contribution of IL-6, TNF-alpha, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice.* J Clin Invest, 2004. **114**(4): p. 582-8.
375. Kobayashi, K., et al., *Cytokine production profile of splenocytes derived from zymosan A-treated SKG mice developing arthritis.* Inflamm Res, 2006. **55**(8): p. 335-41.
376. Hirota, K., et al., *T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis.* J Exp Med, 2007. **204**(1): p. 41-7.
377. Ito, Y., et al., *Gamma/delta T cells are the predominant source of interleukin-17 in affected joints in collagen-induced arthritis, but not in rheumatoid arthritis.* Arthritis Rheum, 2009. **60**(8): p. 2294-303.
378. Wencker, M., et al., *Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness.* Nat Immunol, 2014. **15**(1): p. 80-7.
379. Hirota, K., et al., *Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model.* J Exp Med, 2007. **204**(12): p. 2803-12.
380. Nishimura, K., et al., *Tofacitinib facilitates the expansion of myeloid-derived suppressor cells and ameliorates arthritis in SKG mice.* Arthritis Rheumatol, 2015. **67**(4): p. 893-902.
381. Byun, J.K., et al., *Rebamipide attenuates autoimmune arthritis severity in SKG mice via regulation of B cell and antibody production.* Clin Exp Immunol, 2014. **178**(1): p. 9-19.
382. Murata, K., et al., *MicroRNA-451 down-regulates neutrophil chemotaxis via p38 MAPK.* Arthritis Rheumatol, 2014. **66**(3): p. 549-59.
383. Oh, K., et al., *CP-690550 Treatment Ameliorates Established Disease and Provides Long-Term Therapeutic Effects in an SKG Arthritis Model.* Immune Netw, 2013. **13**(6): p. 257-63.
384. Tsunemi, S., et al., *Molecular targeting of hepatocyte growth factor by an antagonist, NK4, in the treatment of rheumatoid arthritis.* Arthritis Res Ther, 2013. **15**(4): p. R75.
385. Azuma, K., et al., *Suppressive effects of N-acetyl-D-glucosamine on rheumatoid arthritis mouse models.* Inflammation, 2012. **35**(4): p. 1462-5.
386. Bahtiar, A., et al., *The l-Ser analog #290 promotes bone recovery in OP and RA mice.* Pharmacol Res, 2011. **64**(3): p. 203-9.
387. Misaki, K., et al., *Histone deacetylase inhibition alters dendritic cells to assume a tolerogenic phenotype and ameliorates arthritis in SKG mice.* Arthritis Res Ther, 2011. **13**(3): p. R77.
388. Duarte, J., et al., *Modulation of IL-17 and Foxp3 expression in the prevention of autoimmune arthritis in mice.* PLoS One, 2010. **5**(5): p. e10558.
389. Tsunemi, S., et al., *Effects of the novel immunosuppressant FTY720 in a murine rheumatoid arthritis model.* Clin Immunol, 2010. **136**(2): p. 197-204.
390. Nagate, T., J. Kawai, and J. Nakayama, *Therapeutic and preventive effects of methotrexate on zymosan-induced arthritis in SKG mice.* J Vet Med Sci, 2009. **71**(6): p. 713-7.
391. Mullazehi, M., et al., *Anti-type II collagen antibodies are associated with early radiographic destruction in rheumatoid arthritis.* Arthritis Res Ther, 2012. **14**(3): p. R100.

392. Cook, A.D., et al., *Antibodies to type II collagen in early rheumatoid arthritis. Correlation with disease progression.* Arthritis Rheum, 1996. **39**(10): p. 1720-7.
393. Fang, F.C., *Antimicrobial reactive oxygen and nitrogen species: concepts and controversies.* Nat Rev Microbiol, 2004. **2**(10): p. 820-32.
394. Winkelstein, J.A., et al., *Chronic granulomatous disease. Report on a national registry of 368 patients.* Medicine (Baltimore), 2000. **79**(3): p. 155-69.
395. Olofsson, P., et al., *Positional identification of Ncf1 as a gene that regulates arthritis severity in rats.* Nat Genet, 2003. **33**(1): p. 25-32.
396. Hultqvist, M., et al., *Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene.* Proc Natl Acad Sci U S A, 2004. **101**(34): p. 12646-51.
397. van de Loo, F.A., et al., *Deficiency of NADPH oxidase components p47phox and gp91phox caused granulomatous synovitis and increased connective tissue destruction in experimental arthritis models.* Am J Pathol, 2003. **163**(4): p. 1525-37.
398. De Ravin, S.S., et al., *Chronic granulomatous disease as a risk factor for autoimmune disease.* J Allergy Clin Immunol, 2008. **122**(6): p. 1097-103.
399. Gelderman, K.A., et al., *T cell surface redox levels determine T cell reactivity and arthritis susceptibility.* Proc Natl Acad Sci U S A, 2006. **103**(34): p. 12831-6.
400. Hultqvist, M., et al., *Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice.* J Immunol, 2007. **179**(3): p. 1431-7.
401. George-Chandy, A., et al., *Th17 development and autoimmune arthritis in the absence of reactive oxygen species.* Eur J Immunol, 2008. **38**(4): p. 1118-26.
402. Gelderman, K.A., et al., *Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species.* J Clin Invest, 2007. **117**(10): p. 3020-8.
403. Circu, M.L. and T.Y. Aw, *Reactive oxygen species, cellular redox systems, and apoptosis.* Free Radic Biol Med, 2010. **48**(6): p. 749-62.
404. Simon, H.U., A. Haj-Yehia, and F. Levi-Schaffer, *Role of reactive oxygen species (ROS) in apoptosis induction.* Apoptosis, 2000. **5**(5): p. 415-8.
405. Zhang, Y., et al., *ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages.* Cell Res, 2013. **23**(7): p. 898-914.